July 21, 2025

Comments to the United States Department of Agriculture (USDA-APHIS) submitted by the Canadian Biotechnology Action Network (CBAN)

RE: Petition for determination of nonregulated status for blight-tolerant Darling 54 American chestnut (Castanea dentata), Originally Submitted January 17, 2020. Revised with event name correction, August 9, 2024;
USDA-APHIS Draft Environmental Impact Statement;
USDA-APHIS Draft Plant Pest Risk Assessment.

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The Canadian Biotechnology Action Network (CBAN) brings together 15 groups to research, monitor and raise awareness about issues relating to genetic engineering in food and farming in Canada. CBAN members include farmer associations, environmental and social justice organizations, and regional coalitions of grassroots groups. CBAN has almost two decades of experience in researching and monitoring the impacts of genetically modified organism (GMOs), including examining the issues raised by the possible release of genetically engineered trees and documenting incidents of GMO escape and contamination. CBAN is a project on the shared platform of the MakeWay Charitable Society.

Introduction

The Canadian Biotechnology Action Network (CBAN) appreciates this opportunity to comment on the important question of United States Department of Agriculture (USDA-APHIS) deregulation (approval) of the genetically engineered (GE or genetically modified) American chestnut tree "Darling 54", previously known as "Darling 58," for release into the open environment, and to comment on the revised petition (2024) from the State University of New York College of Environmental Studies and Forestry (ESF) and the related revised APHIS Draft Environmental Impact Statement and Draft Plant Pest Risk Assessment.

We contend that, because the intention is open release of this genetically engineered tree for deliberate spreading across the full range of the American chestnut, **the unconfined release of Darling 54 trees represents, by definition, a plant pest risk as a potential invasive.**

We are alarmed that the ESF revision of their 2020 petition for deregulation was necessitated by the discovery of a significant error in the research and development process such that the subject

of the 2020 petition, "Darling 58," was not actually the subject of the tests and analysis documented. We are further alarmed by the dismissive treatment of this mistake and its potential implications. The significantly different, suboptimal GE event "Darling 54" was mistakenly studied for six years. That this fundamental mistake was made and remained unnoticed for so many years supports a conclusion that long-term testing and more rigorous study is required before deregulation can be considered. Furthermore, ESF's revised petition does not address the 2023 reporting, from their research partner The American Chestnut Foundation (TACF), of various performance limitations with the Darling trees. ESF should have withdrawn their petition, and as requested by TACF.[1]

At issue is the precedent-setting request from ESF to release a genetically engineered American chestnut tree for deliberate planting and spread in the open environment. The USDA-APHIS decision is of pressing concern for the future of the American chestnut in both the US and Canada. The impacts of a decision to allow planting of this genetically modified organism (GMO) in the United States would reach across the national border into Canada where the American chestnut (*Castanea dentata*) is protected as an endangered species. The inevitable spread of this GE American chestnut tree into Canada means that a decision to deregulate this GE tree in the United States would be a *de facto* decision to release it in Canada. Releasing this GE tree in the United States would directly affect the environment in Canada.

The release of Darling 54 could threaten the endangered American chestnut in Canada and undermine the future of American chestnut restoration efforts in Canada. The introduction of this genetically engineered tree would also add potential new threats to ecosystems and forests across eastern North America that are already vulnerable and stressed. Any possible negative environmental impacts may not be known for decades or centuries. As stated in the Draft Environmental Impact Statement (dEIS), "planting of Darling 54 American chestnut would not be considered a short-term use of the environment since the trees can live hundreds of years," (page 4-43) and yet APHIS does not consider this long timeframe in relation to the many unknows associated with using genetic engineering, and the Darling 54 event in particular.

The release of this GE tree is contested in both Canada and the US. A decision to allow its use would be made on behalf of all peoples living in the range of the American chestnut, and on behalf of many future generations. The fate of the forests, ecosystems, and landscapes of the range of the American chestnut crosses our national border as well as the territories of many Indigenous peoples who have not been consulted on this issue.

A decision to release Darling 54 is, therefore, a momentous responsibility. Yet, the environmental impact assessment is limited in both the scope and timescale. The wide range of this large-scale release and spread is not considered, and the potential long-term impacts are not assessed.

We urge USDA-APHIS to respond to this petition with the "No Action Alternative" of continued regulation of the Darling 54 American chestnut, rejecting the "Preferred Alternative" to approve the petition to allow open release of this genetically engineered tree.

Overview

The request of the proponents (ESF) is for deregulation to allow release of the genetically modified organism (GMO), now known as "Darling 54", into the open environment, to deliberately spread these genetically engineered (GE) trees across the eastern United States.

- The use of the suboptimal, flawed GE event "Darling 54" poses increased risks over "Darling 58" that are not addressed by ESF in their revised petition.

- The discovery of the identity error whereby researchers were, for six years, experimenting with the wrong GE Darling event, and the indifference of ESF's response, is a worrying indication of carelessness in high-stakes research.

- The field test results, reported in December 2023, from ESF's research partner The American Chestnut Foundation are not noted in the revised 2024 petition and these observations of multiple performance limitations are not discussed.

- Release of this GE tree would have long-term impacts across, and beyond, the natural range of the American chestnut in North America, including in Canada.

- The timescale involved in the life of this GE tree and its progeny and the vast uncertainties involved in its release demand the use of the Precautionary Principle in decision making, to ultimately conclude that this release cannot be permitted.

- The American chestnut restoration efforts underway in both Canada and the US provide hope for the future of wild American chestnuts. These efforts should be supported rather than jeopardized by the release of this genetically engineered tree.

- A decision to permit release of this GE tree would set a dangerous precedent for the deliberate release of other GM plants into the open environment, and other GE trees: if multiple and an increasing number of GMOs are released, the risks of interactions and cumulative environment impacts will increase over time.

The USDA-APHIS analysis is lacking:

- The implications of the mistaken use of Darling 54 in experimentation, an error that remained undetected for six years, are not adequately considered.

- Fundamentally, the timeframe and scope of the risk assessment is too limited and cannot account for the possible future impacts of releasing this GE tree.

- Despite explicitly acknowledging the difficulty of predicting the impacts on biodiversity, the draft Environmental Impact Statement makes conclusions about benefits, without equally addressing risk.

- The draft Environmental Impact Statement underestimates the degree and speed of seed movement, and role of human intervention in the spread of Darling 54.

- The draft Environmental Impact Statement overlooks the potential of existing American chestnut restoration efforts that do not use genetic engineering and does not examine the impacts of a Darling 54 release on those restoration strategies.

We have serious concerns about the risks posed by deregulation of the genetically engineered American chestnut tree, now known to be "Darling 54," to the Canadian environment, to the endangered American chestnut *(Castanea dentata)* in Canada, and to chestnut restoration efforts in both Canada and the United States.

We contend that a decision to release this GE American chestnut tree in the United States would be a *de facto* decision to release the GE tree in Canada. The release of Darling 54 poses risks in the long-term across a wide geography that crosses our national border as well as the territories of many Indigenous peoples who have not been consulted on this issue.

This spread of Darling 54 would occur across many ecosystems and landscapes including those in Canada. The range of the American chestnut tree extends in the east of North America into southern Ontario and is projected to move into the Maritimes due to climate change.[2] However, the tree can also grow outside its range. In fact, the largest American chestnut tree in Canada is growing in the province of Nova Scotia. Sites of American chestnut have been identified and/or initiated by conservationists in eastern Ontario, Quebec, Nova Scotia, Prince Edward Island, and British Columbia. The American chestnut is protected as an endangered species in Canada and is protected under the federal Species at Risk Act[3] and the Government of Ontario's Endangered Species Act.[4]

In Canada, there is existing, promising restoration work led by non-profit conservation groups and teams of many dedicated volunteers, in particular work led by the Canadian Chestnut Council. This work has been underway for decades in a Canadian context, supported by projects to identify wild individuals and break isolation in order to encourage propagation.[5] This work is guided by the American chestnut recovery strategies of the Government of Canada[6] and the Province of Ontario.[7] Good prospects remain for recovery of the American chestnut in Canada using these existing strategies that do not involve genetic engineering. Contamination from the Darling 54 tree, and the implementation of measures to prevent such contamination, could put these important restoration efforts at risk.

The release of this GE tree is contested in both Canada and the US, however an APHIS decision to allow its use would be made on behalf of all peoples living in the range of the American chestnut, and on behalf of many future generations.

The dispersal and spread of the Darling 54, through gene flow and human intervention, cannot be monitored or controlled now and in the future, and the impacts of its release may never be fully known or understood. Yet, the environmental impact assessment is limited in both the scope and timescale. The wide range of this large-scale release and spread is not considered, and the potential long-term impacts are not assessed.

The revisions to the petition and environmental impact statement are not sufficient

We are commenting on the revised ESF petition and revised APHIS Draft Environmental Impact Statement and Draft Plant Pest Risk Assessment. The revisions to the petition from ESF and the subsequent revisions to the risk assessment documents were triggered by a recently discovered, significant mistake made by ESF researchers whereby researchers had mistakenly used the GE event "Darling 54" in their tests instead of the event "Darling 58" which was named in the 2020 petition. Until October 2023, the Darling tree was understood to be Darling 58.[8] The GE tree under study and subject to the petition is actually the suboptimal, flawed GE event called Darling 54.

APHIS describes that, "SUNY-ESF submitted a revised petition incorporating name corrections and providing clarification regarding the plant, additional updates and research findings related to molecular characterization, and other minor technical corrections."[9] However, we are alarmed by the significant error made by ESF in 2016 that led to years of experimental research with the wrong genetically engineered Darling event. This is not simply a labelling error that can be rectified by just renaming "Darling 58" as "Darling 54." We are very concerned that ESF, and APHIS, are not considering the full implications of this mistake including the fact that the error remained undiscovered for so many years as research continued. This fundamental error and its longevity are indications of poor research practice and underline the potential for unexpected errors that could have significant environmental consequences.

The mistaken use of Darling 54 instead of Darling 58 dates back to 2016. Arguably, rigorous research of Darling 58 would have more quickly revealed that the tree was, in fact, was Darling 54.

This mistake also raises concrete environmental risk concerns that are not addressed in the petition. The Darling 54 event was not the chosen event for research and development because of the suboptimal location of the OxO gene that introduces new unknowns. 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. This means that the molecular characterization offered in the initial petition was wrong.

The petition revisions made by ESF are a superficial response to this fundamental error. Of the insertion location, ESF simply writes that, "The transgene insertion site in Darling 54 matches the sequence of a known gene in other plants (Sal1). This gene has not been studied in chestnuts, but based on studies of Sal1 and similar genes in other plants, interruptions at this site are unlikely to result in plant pest risks, especially in the context of larger genomic changes that can often result from hybrid breeding," (page 4) and, "The initial submission of this Petition (January 2020) referred to Darling 58 instead of Darling 54. These two lines were produced at the same time, using the same transgenes in the same genetic background, so they express the same protein products." (page 5) This is not sufficient examination of the potential implications. Darling 58 was chosen for research and development over Darling 54 for obvious reasons that are now glossed over.

Reports of new field test results are unaddressed

We draw your attention the fact that there have been important developments in this file since the preparation of the 2020 petition that are not addressed in the revised petition. In particular, the petition does not address new observations from Darling field tests brought forward by ESF's research collaborator The American Chestnut Foundation (TACF).

In December 2023, TACF announced their observations of "significant performance limitations" with the Darling trees. The TACF summarized that they saw, "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."[10] These observations were not acknowledged and explicitly addressed by ESF in their revised petition and remain unresolved in the public discourse.

These results, not yet published in peer reviewed literature, were important enough to lead TACF to conclude that the Darling tree is "unsuitable as a restoration tree" and to decide to withdraw their support from this petition.[11]

Assessing the request to release Darling 54 requires use of the Precautionary Principle

This petition request is for the first-ever intentional release of a genetically engineered plant into the open environment. Such a decision would be a national, North American, and global first. The important precedents that would be set by this decision need to be considered because of their significant environmental and social implications.

The question of this release is complex and profound because the impacts are far-reaching in time and space. The timescale involved reaches beyond our sight to impact many future generations, and the release has the potential to impact ecosystems at the outer limit of where the American chestnut can grow in North America.

The question requires decision makers to employ the Precautionary Principle which allows for protective action where there is scientific uncertainty about risks. In this case, in addition to the uncertainties surrounding genetic engineering, environmental assessment is complicated by our ignorance of forest ecology. In the context of the climate and biodiversity crises, the stakes involved are further enhanced, where the introduction of this GE tree could add yet another stress on vulnerable species and ecosystems.

A comprehensive assessment of the environmental impacts of planting genetically engineered trees in the wild is not possible given the complexity of trees and their long life, and the complexity of forest ecosystems and the size of the habitats involved.[12] The limitations of our tools and knowledge for assessing the environmental risks of US-wide or continent-wide open release of this GE tree need to be explicitly acknowledged.

The Precautionary Principle also demands consideration of the need for the use of this technology in relation to the alternatives. In the case of the American chestnut, there are significant ongoing restoration efforts in both Canada and the US that show potential for success while also avoiding the unique and complex risks presented by the use of genetic engineering.

The following features of the proposed release of Darling 54, as discussed below, should trigger use of the Precautionary Principle:

- The timescale reaches far into the future;
- The scope and geographical spread of potential impacts are wide;
- There are vast uncertainties and unknowns;
- Impacts could be irreversible and profound;
- Spread cannot be monitored and controlled;
- Release will threaten wild American chestnut restoration efforts.

The timescale and scope of the APHIS assessment is inappropriately constrained

If released, the future of Darling 54 would be entangled with the future of many ecosystems and landscapes, and many generations of species, including humans. The long timeframe and wide scale of this release should trigger use of the Precautionary Principle. Instead, the analysis presented in the USDA APHIS Draft Environmental Impact Statement (dEIS) is based on a very limited timescale and scope that overlooks potential, widespread future risks.

The dEIS states that, "Planting of Darling 54 American chestnut would not be considered a short-term use of the environment since the trees can live hundreds of years" (page 4-43) and the agency says that it is tasked with considering both "short- and long-term effects" (page 4-15). However, the assessment does not consider the long-term effects. Despite stating that, "Impacts/effects may occur soon after the Agency decision or occur later in time" (page 4-16) the assessment does not fully quantify and consider "later in time."

At the outset, the assessment accepts the intention of the petitioner (ESF) as "ecological restoration" along with their proposal that "initial distribution will consist of long-term research plots and relatively small-scale horticultural plantings and will focus on areas where there are surviving small remnant American chestnut populations." (page vi) However, at the same time, the dEIS refers to future larger scale planting in forests as is explicitly designed in the purpose of the Darling project: "Restoration with Darling 54 American chestnut is expected to occur on long-term research plots and relatively small-scale public horticultural lands before being planted on a larger scale in eastern forests and utilize similar resources as other forest trees." (page 4-43) The assessment states that, "Without aggressive restoration efforts, requiring considerable effort and coordination at landscape scales, it may require centuries before American chestnut becomes a significant presence in the landscape." (p v). However, the proponents explicitly propose aggressive restoration efforts and, since their petition of 2020, have entered into non-exclusive licensing agreements with at least one company.[13]

In assessing the economic impacts, the dEIS analysis concludes with an "expectation" that the Darling 54 will not be used in commercial plantations in the "foreseeable future": "Darling 54 American chestnut's attributes have yet to be established for timber production and so the risk of using Darling 54 American chestnut in a commercial venture is likely too high for adoption in the foreseeable future."(page vi) This conclusion leaves commercial production in the future as an open question, with the long-term seemingly not considered relevant to the assessment of economic impacts. More importantly, since the ESF petition was submitted in January 2020, ESF has established a nonexclusive licensing agreement with the privately held company "American Castanea" (now Silvabio), founded in 2022, which, in 2023, was reported to be planting and propagating the GE trees in New York State.[14] In the 2020 petition, ESF described, "Restoration efforts will primarily be managed by The American Chestnut Foundation (TACF), a non-profit organization which is a supporter and collaborative partner with ESF," but this was reworded in the revised 2024 petition to, "Restoration efforts in conjunction with ESF will be managed and supported by non-exclusive partnerships with various entities, potentially including non-profit organizations, other universities, companies, and state & federal agencies."(page 5)

The dEIS acknowledges that any long-term impacts would not be measurable for decades or longer and yet makes conclusions about these impacts. On balance, there also appears to be a deemphasis on risks, where benefits are mentioned without equally commenting on risks. For example, the statement is that made that, "Any long-term benefits wouldn't be measurable for decades or longer. As such, no effects are expected to listed and proposed T&E [Threatened and Endangered] species and critical habitat where Darling 54 American chestnut would be planted." (page 52) while it is equally, or more so, that any long-term risks would not be measurable for decades or longer.

The scope of analysis is also constrained to impacts that are "reasonably foreseeable." The scope of analysis is partly described thus:

> "Pursuant to NEPA, effects considered are those that are reasonably foreseeable and have a reasonably close causal relationship to the petition decision. Effects may occur soon after the Agency decision or occur later in time. Potential effects include ecological (such as the effects on natural resources and on the components and functioning of affected ecosystems), historic, cultural, social, or effects on public health. Economic effects, such as those on employment or markets, may also be considered. Effects include those resulting from actions that may have both beneficial and detrimental effects, even if on balance the agency believes that the effect will be beneficial."(page 4-16)

However, the core challenge is future impacts that may be beyond sight.

Furthermore, the assessment does not consider the precedent that would be set by a decision to allow the first GE plant for release into the wild, i.e. How many GE trees or other GMOs will government agencies approve for release into the open environment, and for what purpose? Currently, each GMO release is assessed individually, on a case-by-case basis, without reference to others or attention to possible cumulative impacts. As discussed by Jack A. Heinemann et al., the risk relating to the use of gene technologies correlates with scale, where harm increases with the increased use of the technology and exposure to it.[15]

In fact, the GE American chestnut has been described by some supporters as a "test tree" or "poster child" that could be used to build public support for the use of other genetically engineered trees for industrial purposes.[16] There is a range of research and development into genetically engineering tree species for commercial use,[17] including research being pursued by ESF on disease and pest resistance in other hardwood tree species such as elm.[18]

The possible impact of this one decision on the future of GE trees is critical because, as concluded by Fundación Ambiente y Recursos Naturales (FARN); Huni Kui Peoples' Federation of Acre, Brazil; Indigenous Environmental Network; Ecoropa; Global Forest Coalition; Global Justice Ecology Project; Biofuelwatch; and the Canadian Biotechnology Action Network, "Genetically engineered trees are a threat to a sustainable future. Genetic engineering provides a distraction from real solutions and its deployment would pose a concrete danger to forest ecosystems."[19]

There are vast uncertainties and unknowns relating to the release of Darling 54

The Precautionary Principle is available to assist decision-making in the face of uncertainties and unknowns. The dEIS acknowledges it is difficult to predict what impacts the Darling 54 American chestnut will have on forest biodiversity. This inability to predict the impacts should trigger the use of the Precautionary Principle and lead to a determination to regulate the Darling 54 (not approve its release) in order to avoid environmental harm.

Forest ecosystems are highly complex and poorly understood, and this incredible complexity increases the unknowns and uncertainties of introducing a GE tree. Assessing how the release of Darling 54 would affect other trees, understory plants, insects, soils, fungi, and wildlife over time, would require a far better understanding of forest ecology than we currently have.

Furthermore, it is not possible to assess the risks because we do not know what will happen in complex ecosystems that are subject to climate change, over multiple generations of American chestnut trees which can live for over 200 years. The impacts of release on ecosystems are unknown, and cannot be known, until they are observed in the wild over decades or centuries.

In the face of the acknowledged inability to predict the impacts, the dEIS, instead, makes conclusions of benefit. For example, "While it is difficult to predict what impacts Darling 54 American chestnut will have on forest biodiversity, in the long term if American chestnut were to become a dominant species again, it is reasonable to believe there are likely to be positive impacts on the biodiversity of animal species…"

The conclusions made in the assessment about the lack of impacts on biodiversity are weak and qualified. Such qualifications are necessary given the perfunctory attention paid to these questions in the assessment and our inability to know the impacts in the long-term. Statements made about the possible negative impacts on biodiversity simultaneously recognize uncertainty and gloss over it. For example:

- **"**While **it is difficult to predict what impacts Darling 58 American chestnut will have on forest biodiversity**, especially since the overall ecosystem has changed since American chestnut disappeared from the landscape, **it is reasonable to believe** that if Darling 58 American chestnut shows enhanced tolerance to chestnut blight and the trees are able to establish and spread, in the long term it will have positive impacts on increasing the biodiversity of animals and micro-organisms while decreasing the abundance of some tree species such as oaks (Paillet 2002)." [bolding added] (page 3-11)
- "As discussed throughout this EIS, the impacts of a determination of nonregulated status for Darling 58 American chestnut are **unlikely to be adverse**." [bolding added] (page 4-44)

With so much at stake, these conclusions are not robust enough to allow for a decision to deregulate.

There are uncertainties resulting from genetic engineering

The processes involved in genetic engineering commonly result in unintended changes to DNA and traits.[20] Altering or introducing genes can result in changes, not only to the target gene(s) but also elsewhere in the genome, in unexpected, often surprising and unpredictable ways that can have profound impacts on the organism.[21] For example, unintended traits are commonly observed in commercialized GE crops.[22]

With long-lived organisms such as trees, detecting unintended traits is more challenging. The dEIS states that, "According to information submitted by the petitioner and reviewed by APHIS, Darling 54 American chestnut is phenotypically and biochemically comparable to conventional American Chestnut (ESF 2024)," (page 47) however it is not possible to conclude from the studies available that the GE tree is comparable to non-GE American chestnuts. Further study and observation over time may find meaningful differences with implications for biodiversity.

If the Darling 54 is released, the GE tree may be later found to exhibit characteristics that are not comparable to wild American chestnuts. Genes do not function as isolated units but interact with each other and their environment in complex ways that are not well understood or predictable. Changes made to any of the genes involved can have far-reaching impacts, even on seemingly unrelated traits. Unexpected traits can be the product of gene-environment interactions and may only become apparent, for example, during times of environmental stress such as drought.

The intended GE trait of blight tolerance could also fail to function over time, and the impact of the location of the transgene in Darling 54 on the stability of the blight tolerant trait or other traits is not examined. The studies submitted to as part of the petition for approval offer limited information about the future performance of the GE trait however The American Chestnut Foundation reported inconsistent blight resistance in 2023.[23] The tests have all been on young trees grown in the laboratory and short-term field tests, even though it is known that younger trees are naturally more resistant to the blight.

ESF acknowledges a level of uncertainty due to selective pressure: “Since this transgene product does not act against the fungus (Section 4), but rather allows the tree to tolerate infections without fatal damage (Section 8.1), Darling chestnuts are more appropriately termed blight *tolerant* than blight *resistant* (Section 6.3). This tolerance mechanism **should** provide a uniquely stable plant defense, as the likelihood of a pathogen evolving to overcome a tolerance trait is minimized in the absence of a strong selective pressure (Section 5.3)” [bolding added].

American chestnut trees can live for over 200 years and the performance of the GE trait or the expression of new characteristics can be affected by environmental conditions - such as drought, flood, heat, pests - experienced over its lifespan as well as by basic changes associated with aging. Yet there has been no study through the full lifecycle of Darling 54 trees or, further, with multiple generations. Instead, the petitioner relies on studies of three-year-old Darling 54 trees along with data from studies using earlier Darling research. This data is not sufficient for risk assessment and is not sufficient to assess the stability of the GE blight-tolerant trait over time.

The impacts of release may be profound and irreversible

Just as the timescale of the impacts needs to be considered, so does the nature of the impacts i.e. what type of impacts, and of what significance. The release of the GE American chestnut tree is likely to be irreversible. **The nature of the release is one that cannot be monitored or controlled, and may be difficult or impossible to recall.**

The dEIS states the need to consider irreversible impacts in the following way: “An irreversible or irretrievable commitment of resources refers to impacts on or losses of resources that cannot be recovered or reversed. Irreversible commitments of resources are those that cause either direct or indirect use of natural resources such that the resources cannot be restored or returned to their original condition. Irreversible impacts entail the loss of future options, and applies primarily to the use of non-renewable resources such as fossil fuels, and resources that are renewable only over long timespans. Irretrievable is a term that involves the loss of productive value or use of resources. For example, certain opportunities can be foregone during the conduct of a proposed action, during which a resource cannot be used. These actions may be reversible or temporary, but the utilization opportunities foregone during the action are irretrievable.” (page 4-43) We contend that the impact of the Darling 54 on the future of wild American chestnut in Canada could be irreversible, where the **restoration of wild American chestnuts is lost as a future option**.

Distribution and spread is unknown, and will be unmonitored and uncontrolled

Release of the Darling 58 in the United States would ultimately result in unknown, unmonitored, and uncontrolled (and uncontrollable) spread and distribution of this genetically engineered tree across the US and into Canada.

The spread and distribution of Darling 54 may be much faster than assumed in the dEIS assessment and could mean that the Darling 54 becomes a plant pest risk. The USDA-APHIS

concludes that, “The American chestnut is not considered an invasive, fast colonizing tree, and the OxO gene will not change these traits. Therefore, Darling 54 American chestnut is not expected to invade or alter critical habitat in ways that would be detrimental to T&E species.”(pages 47-48) However, the Darling 54 could become invasive in Canada.

The dEIS states that “Potential effects on the environment will depend on the success of Darling 54 chestnut to survive and spread over time” (page 4-16) but the analysis of potential impacts relies on incorrect assumptions about limited distribution in the short and mid-term. We argue that the analysis makes a number of incorrect assumptions including that, “Areas that are not intentionally planted with blight-tolerant chestnuts will likely remain without chestnuts for decades or longer (ESF 2024)”(page 3-10/11) and that “Darling 54 American chestnut could effectively be excluded from critical habitat if needed.”(page 48)

Critically, this projection of limited distribution (dependent on human intervention) conflicts with the intended use of the Darling 54 as restated by APHIS in Appendix 1 of the dEIS that, “Darling 54 American chestnut is intended to be used as a restoration tree to establish and colonize much of the eastern United States....” (page 47). In their petitions, ESF states that, if the Darling trees are granted nonregulated status, “they will be made available for not-for-profit distribution to the public, and to groups including private, indigenous, state, and federal restoration programs, depending on the goals and preferences of these various groups.”(ESF 2024 petition, page 5) The proponents stress that the GE tree is not patented “so as not to impede any American chestnut distribution or restoration efforts.” They also stress the role of “citizen scientists” and say that the efforts towards outcrossing Darling 54 with wild chestnuts will “rely on the public” and “Researchers will continually seek feedback, but **the public will ultimately be able to propagate these trees, share them and plant them as they wish**” [bolding added]. In our view, these statements, along with varying experience with the spread of AC (please see below), contradict the ESF argument that “successful colonization by transgenic chestnuts in areas beyond where they are intentionally planted will be relatively slow and manageable, depending on the preference of land managers.” (ESF petition, page 5)

Since there is no apparent intention for government monitoring of its distribution, the spread of Darling 54 cannot be assumed to follow any stated plan of the proponents. This is especially the case because ESF’s plans have already changed from their 2020 petition where restoration efforts were to be primarily managed by The American Chestnut Foundation to now being “managed and supported” by partnerships with “various entities” including companies.(Page 5) In fact, the distribution and the spread of Darling 54 is unknown, cannot be fully unmonitored, and cannot be fully controlled.

Our experience with the use of GMOs in agriculture, and with invasives, demonstrates the difficulty of managing and recalling GMOs.[24] A clear lesson from this experience is the role of human behaviour or human error in the unwanted spread of GMOs. In some cases, the cause of unintended GMO escape remains undetermined. The dEIS does not examine the upper limits of how far and wide, and how quickly, the Darling 54 could be distributed and spread.

Monitoring and managing the spread of this GE tree in the US would be important in order to track the risk of contamination and prevent any contamination into Canada. Such monitoring

would be also vital to observing and tracking any potential adverse environmental impacts, and to maintaining an ability to recall the GE tree if impacts are observed. If monitoring and management of spread is not possible, including because it is not enforceable, then the risks of Darling 54 increase. Because the American chestnut is such a long-lived organism and its spread may be slow, any adverse impacts may only be observed over decades or centuries by which time the GE tree may be widely dispersed and recalling it may not be possible.

Release would threaten an endangered species in Canada and jeopardize restoration

Release of the Darling 54 in the United States would threaten the American chestnut in Canada which has legal protection as an endangered species.. Furthermore, northern spread of this GE tree from the United States would undermine promising restoration efforts in Canada that are supported by provincial (Ontario) and federal government recovery strategies.

APHIS repeats the petitioner's description that their intention is "ecological restoration" but we contend that the Darling 54 cannot be understood as a "restoration tree." A genetically engineered tree would replace rather than restore the American chestnut. Furthermore, the The American Chestnut Foundation has also now stated their 2023 opinion that the Darling 58/54 is "unsuitable as a restoration tree" due to "significant performance limitations."[25]

The USDA repeats ESF's depiction of the Darling 54 as a "restoration tree" but does not assess the restoration claim. Though not examining the ability of the tree to meet this stated goal of restoration, the general assumption of restoration appears embedded in the assessment. For example, the dEIS repeats the ESF characterization that that, "Darling 54 American chestnut was developed with the intent of restoring a native tree to its former range", however, from a risk assessment point of view, this statement should be written: "The genetically engineered American chestnut was developed with the intent of release and spread in the open environment."

The dEIS recognizes the reality that even if the Darling 54 works as intended to be successfully and stably tolerant to the blight *Cryphonectria parasitica* across generations, it will face the same disease pressures as any other American chestnut: "Its attributes have yet to be established and it is susceptible to other diseases such as ink disease caused by *Phytophthora cinnamomi* (ESF 2019)." (page vi) These disease pressures may sabotage the intended use of Darling 54. Furthermore, the dEIS states, "As the climate and ecology of the eastern forests have changed in the last hundred years it is unknown whether Darling 54 American chestnuts will ever regain the dominance it exhibited in the nineteenth century. (page vi) Furthermore, such efforts to genetically engineer pathogen resistance are likely to be unsuccessful over time simply because pathogens quickly evolve to overcome plant defenses.[26] The ability of the Darling 54 to function as intended is therefore not assured, and yet its release would disadvantage, and could sabotage, other restoration strategies.

The APHIS assessment does not acknowledge and consider the potential of existing restoration efforts in the US and Canada. We do not agree with the conclusion that, if release of Darling 54 is not authorized, "American chestnut would remain stumps and small understory shrubs (Elliott

and Swank 2008; Dalgleish et al. 2015b) in the forests of the eastern United States where populations persist." (page 4-19) The dEIS states that, "American chestnuts are still found wherever American chestnut was a canopy tree before the blight" but adds that, "They persist as stumps and small trees, often multi-stemmed, as a result of the blight, and no longer occupy a dominant canopy position." (Page 4-19) However, there are naturally blight tolerant individuals in both countries and these trees hold potential for restoration. In fact, **there is a greater proportion of large American chestnut trees and trees with reproductive capacity in Canada than in the US**[27] which reinforces the need to protect current restoration efforts in Canada.

There is ongoing restoration work in both Canada and the US that relies on remaining wild American chestnuts and does not use, and does not support the use of, genetic engineering. In fact, there is hope in Canada that communities of conservationists will succeed in their long-term work to establish plots of naturally blight tolerant American chestnuts. This vision for restoration of the American chestnut without genetic engineering is shared by many communities across the US and Canada and has been supported by decades of work by dedicated volunteers who have been identifying and studying blight-tolerant individuals in the wild

These efforts would be threatened by the release of a genetically engineered American chestnut tree that would spread and could crossbreed.[28] The release of this GE tree in the United States would pose a major management concern for Canadian restoration efforts. Implementing measures to stop gene flow and the movement of Darling 54 trees, including due to illegal importation, could, at the very least, slow down restoration efforts. A Darling 54 release in the US will likely spread across the range of American chestnut into Canada and the Darling 54 may also be planted and successfully grow outside its natural range.

The dEIS mentions that the spread of Darling 54 could take centuries. This same timeframe should be applied to considering the potential for existing non-GE restoration efforts. **The work of chestnut restoration demands a long-term commitment, and it is too soon to abandon this important work to the release of a genetically engineered tree.** The release of Darling 54 could remove this possible future option of restoration with wild American chestnuts and could facilitate the extinction of the American chestnut.

Conclusion

Rather than restoring the American chestnut, the release of this genetically engineered tree would threaten the future of the American chestnut and could place other species or the balance of forest ecosystems in North America at risk. The release of Darling 54 is explicitly designed to breed with and replace remaining wild American chestnuts. The genetically engineered Darling 54 tree is currently illegal in Canada and its spread would represent a direct threat to the wild American chestnut which has legal protection in Canada as an endangered species. Ultimately, instead of securing a future for the American chestnut, the Darling 54 could sabotage it.

We urge USDA-APHIS to reject this request from ESF and respond with the No Action Alternative of continued regulation.

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