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FW: Petition for Exemption from Regulation Under FIFRA for Oxalate Oxidase Gene in Transgenic American chestnut (Castanea dentata)
To: "CMS.OEX" <cms.oex@epa.gov>

Reading file

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Subject: Petition for Exemption from Regulation Under FIFRA for Oxalate Oxidase Gene in Transgenic American chestnut (Castanea dentata)

Dear Administrator Regan:

On behalf of the State University of New York College of Environmental Science and Forestry (ESF) and The American Chestnut Foundation (TACF), we submit their joint petition requesting the U.S. Environmental Protection Agency exercise the discretion granted by section 25(b)(2) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and initiate rulemaking to exempt from the requirements of FIFRA the Oxalate Oxidase (OxO) gene in transgenic American chestnut (*Castanea dentata)* and sexually compatible species. The exemption is appropriate because, as demonstrated by the enclosed petition, OxO poses a low probability of risk to the environment, and is unlikely to cause unreasonable adverse effects to the environment even in the absence of regulatory oversight under FIFRA.

The American chestnut tree was rendered nearly extinct by the middle of the last century due to the ravages of the highly destructive, invasive, fungal species, *Cryphonectria parasitica,* which causes chestnut blight. Some four billion trees were killed, changing forever the character of eastern U.S. forests and their ecosystems. Now, the addition of a single, common wheat gene — OxO — to the American chestnut will permit the restoration of this native tree species to its natural range by enabling it to coexist with (not kill) the chestnut blight fungus, without adverse effects on people or the environment. Petitioners plan to help lead public and private efforts over time to restore the American chestnut to its natural range throughout eastern forests. This goal has received wide support from public enthusiasts, private philanthropists, and environmental conservation groups.

Petitioners urge EPA to consider this petition in parallel with ESF's pending pesticide registration application for the OxO gene in the Darling 58 American chestnut and the associated pending petition for an exemption from the requirement for a tolerance for residues in food. 87 Fed. Reg. 16,470 (Mar. 23, 2022). Petitioners also ask the Administrator to take formal notice of both the Animal and Plant Health Inspection Service's ongoing review of ESF's petition for nonregulated status for the Darling 58 tree under the Plant Protection Act, *see,* 87 Fed. Reg. 67,861 (Nov. 10, 2022), and the U.S. Food and Drug Administration's evaluation of Darling 58 chestnuts under FDA's Plant Biotechnology Consultation Program (BNF185).

Respectfully,

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State University of New York College of
Environmental Science and Forestry

THE
AMERICAN
CHESTNUT
FOUNDATION®

January 18, 2023

Via Electronic Mail and Overnight Courier

The Honorable Michael Regan
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Ave NW
William Jefferson Clinton Building
EPA East Room 1309
Washington, DC 20004

Re: Petition for Exemption from Regulation Under the Federal Insecticide, Fungicide, and Rodenticide Act Pursuant to 7 U.S.C. § 136w(b)(2) for Oxalate Oxidase Gene in Transgenic American chestnut (*Castanea dentata*)

Dear Administrator Regan:

The State University of New York College of Environmental Science and Forestry (ESF) and The American Chestnut Foundation (TACF) hereby respectfully submit their joint petition pursuant to section 4(e) of the Administrative Procedure Act, 5 U.S.C. § 553(e), requesting that the Administrator of the U.S. Environmental Protection Agency exercise the discretion granted by section 25(b)(2) of the Federal Insecticide, Fungicide, and Rodenticide Act, 7 U.S.C. § 136w(b)(2) (FIFRA), to initiate rulemaking to exempt from the requirements of FIFRA the Oxalate Oxidase (OxO) gene in transgenic American chestnut (*Castanea dentata*) and sexually compatible species.

The American chestnut was rendered nearly extinct by the middle of the last century due to the ravages of the highly destructive, invasive, fungal species, *Cryphonectria parasitica*, which causes chestnut blight. Some four billion trees were killed, changing forever the character of eastern U.S. forests and their ecosystems. Now, the addition of OxO — a single, common wheat gene — to the American chestnut will permit the restoration of this proud native tree species to its natural range by enabling it to coexist with (not kill) the chestnut blight fungus, and without adverse effects on people or the environment. Petitioners plan to help lead public and private efforts over time to restore the American chestnut to its natural range throughout eastern forests. This goal has received wide support from public enthusiasts, private philanthropists, and environmental conservation groups. The exemption is appropriate because, as demonstrated by the enclosed petition, the OxO gene in the American chestnut is of a character which is unnecessary to be subject to FIFRA in order to carry out the purposes of that act.

Petitioners request that the Administrator consider this petition in parallel with ESF's pending pesticide registration application for the OxO gene in the Darling 58 American chestnut (under 40 CFR Part 152, et seq.) and the associated pending petition for an exemption from the requirement for a tolerance for residues in food under 40 C.F.R. Part 180. 87 Fed. Reg. 16,470 (Mar. 23, 2022). Petitioners also ask the Administrator to take formal notice of both the U.S. Department of Agriculture Animal and Plant Health Inspection Service's (APHIS) ongoing review of ESF's petition for nonregulated status for the Darling 58 tree under the Plant Protection Act, *see*, 87 Fed. Reg. 67,861 (Nov. 10, 2022), and the U.S. Food and Drug Administration's (FDA) evaluation of Darling 58 chestnuts under FDA's Plant Biotechnology Consultation Program (BNF185).

Respectfully,

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BEFORE THE U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C.

IN RE OXALATE OXIDASE GENE IN THE AMERICAN CHESTNUT AND SEXUALLY COMPATIBLE SPECIES STATE UNIVERSITY OF NEW YORK COLLEGE OF ENVIRONMENTAL SCIENCE AND FORESTRY, and THE AMERICAN CHESTNUT FOUNDATION, Petitioners	EPA Dkt No. __________

Petition for Exemption from Regulation Under the Federal Insecticide, Fungicide, and Rodenticide Act Pursuant to 7 U.S.C. § 136w(b)(2)

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January 18, 2023

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I. REQUESTED RELIEF

Petitioners the State University of New York College of Environmental Science and Forestry (ESF) and The American Chestnut Foundation (TACF), pursuant to section 4(e) of the Administrative Procedure Act, 5 U.S.C. § 553(e), hereby petition the Administrator of the U.S. Environmental Protection Agency (EPA) to exercise the discretion provided by section 25(b)(2) of the Federal Insecticide, Fungicide, and Rodenticide Act, 7 U.S.C. § 136w(b)(2) (FIFRA or the Act), to exempt from the requirements of FIFRA the Oxalate Oxidase (OxO) gene in transgenic American chestnut (*Castanea dentata*) and sexually compatible species. The addition of this single wheat gene to the American chestnut enables this once ubiquitous but now functionally extinct native tree species to coexist with the otherwise highly destructive, invasive, fungal species *Cryphonectria parasitica*, which causes chestnut blight. With blight-tolerant trees, Petitioners plan to help lead public and private efforts over time to restore the American chestnut to its natural range throughout eastern forests and help restore those diminished ecosystems.

Specifically, Petitioners propose the following provision be added to 40 C.F.R. Part 174, Subpart B:

> <u>§ 174.26 Exemptions for Individual PIPs of a Character not Warranting FIFRA Regulation.</u>
>
> The following specific plant-incorporated protectants are exempt from the requirements of FIFRA, other than the requirements of § 174.71:
>
> > (a) Oxalate oxidase gene present in the transgenic American chestnut tree (*Castanea dentata*) and sexually compatible species.

Petitioners request that the Administrator consider and decide this petition in parallel with ESF's pending pesticide registration application for the OxO gene in the Darling 58 American chestnut (under 40 C.F.R. Part 152, *et seq*.) and the associated pending petition for an exemption from the requirement for a tolerance for residues in food under 40 C.F.R. Part 180. 87 Fed. Reg. 16470 (Mar. 23, 2022).

II. PETITIONERS

ESF in Syracuse, New York is the home of the American Chestnut Research and Restoration Project. Led by Drs. William A. Powell and Andrew E. Newhouse, the mission of the restoration project is to conduct basic and applied research that will lead to the development of a blight-tolerant American chestnut tree and, working with partners, to reintroduce a population of these tolerant trees into forest ecosystems of New York and elsewhere in the eastern United States representing the American chestnut's natural range. Initial research on this project commenced in 1990. The project has evolved from basic research into a multifaceted endeavor which includes such areas as the identification of plant pathogen resistance-enhancing genes, the development of American chestnut tissue culture, field testing chestnut trees from tissue culture, public participation through the identification of rare remnant survival chestnut trees, collection and exchange of viable nuts, establishment of large restoration plantations throughout New York State, and addressing the necessary regulatory aspects of reintroducing transgenic restoration trees to the environment. *See*, https://www.esf.edu/chestnut/.

The American Chestnut Foundation (TACF) is a non-profit section 501(c)(3) conservation, education, and scientific organization with the core mission to restore the American chestnut to its native range. Based in Asheville, North Carolina, TACF was founded in 1983 by scientists seeking to develop a blight-resistant American chestnut tree through a backcross breeding program involving the naturally blight-resistant Chinese chestnut. That program continues today, supported by its 16 state chapters, a flagship research farm in Meadowview, Virginia, more than 500 breeding orchards managed by dozens of members and public and private organizations around the United States. TACF is funded primarily through private philanthropy and enjoys more than 5,000 members and supporters, which is remarkable for a non-profit focused on the rescue of a tree species. TACF has a very loyal and generous donor base with a membership retention rate nearly double the national average. This demonstrates the power and durability of this mission. TACF is a proud supporter and partner of the ESF American Chestnut Research and Restoration Project. *See*, https://acf.org/.

III. SUMMARY OF PETITION

Chestnut blight was inadvertently introduced to the United States on imported trees before the turn of the last century, and then spread rapidly and decimated practically all natural stands of the American chestnut by the 1950s. Before the blight, the American chestnut was a dominant forest canopy species in parts of the eastern United States. The loss of the tree radically changed both the forest ecosystems of the eastern United States from Maine to Georgia, and the many human communities that developed around and depended on those forests. The addition of the OxO gene to the American chestnut will make it possible to begin reintroduction of the tree throughout its natural range and help, over time, to restore those forest ecosystems to a more natural state.

As demonstrated by this petition, the OxO gene in the American chestnut is of a character which is unnecessary to be subject to FIFRA because the restoration of the transformed American chestnut does not present appreciable risks to human health or the environment even in the absence of regulatory oversight under FIFRA. 7 U.S.C. § 136w(b)(2).

- Restoration of the American chestnut to its natural range would bring certain environmental, cultural and economic benefits.

- The OxO gene added to the American chestnut is naturally present in a wide variety of plants safely used as food by humans, insects and other animals today and over eons. As might be expected given this history, controlled studies have demonstrated no substantial changes or ill effects on relevant forest species from directly or indirectly consuming or being exposed to chestnut leaves, nuts, pollen or other tissues from transformed trees.

- Likewise, the addition of the OxO gene does not negatively impact the morphology, growth patterns or other characteristics of the American chestnut. Indeed, compared to introducing a single, well understood gene to the tree, it would be far more complex to predict and identify the effects on plant physiology and host ecosystems from traditional crossing with Asian chestnut species, which replaces thousands of native genes with those from non-native species.

- The restoration of the American chestnut with OxO-containing trees is unlikely to drive evolutionary changes in the parasitic chestnut blight fungus to overcome the tree's new "defense" mechanism. The elegance of the mechanism is that OxO acts only to mitigate the physical damage to tree tissues caused by the fungus and does not directly affect the fungus itself. Repeated observations show that the fungus continues to thrive even on the transformed trees. The tree and fungus coexist, much as they do in the native range of the fungus (eastern Asia). Thus, it is more precise to describe the transgenic American chestnut as "blight tolerant." Given the absence of selective pressure on the blight fungus, the protection afforded by the transgene to the American chestnut can be expected to be durable over generations.

- The OxO gene is inherited in a simple and predictable fashion by approximately half the offspring in a cross between a transgenic and a wild non-transgenic tree. Termed "hemizygous" inheritance, this is expected from a single-copy gene found on one chromosome and has been observed over at least four generations in controlled crosses with transgenic pollen. This also means that transgenic trees will continue to produce wild-type blight-susceptible offspring, further reducing the chance that the fungus will evolve resistance to the detoxification mechanism of OxO.

- The reintroduction of the American chestnut will restore balance and diversity to its native ecosystems and will not otherwise damage those systems. There is no risk to remaining wild chestnut populations. Currently, there are essentially no functional populations of mature wild American chestnut left to displace. Moreover, a stock of purely wild-type chestnuts will always remain because the OxO trait will be inherited by only half of all transgenic offspring.

- The reintroduction of the American chestnut will not create forests of clones. These are not crops. Trees of the same species naturally evolve unique regional traits well adapted to make them successful in the particular climactic and other competitive circumstances of their respective regional ecosystems. To be successful throughout the American chestnut's range, trees with the OxO trait will be crossed with surviving trees from across the region, so that offspring inherit and pass on their unique genetic diversity evolved over ages. Such diversity is already being incorporated into controlled pollination breeding under permits from the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS).

- Full FIFRA oversight of OxO in the Darling 58 American chestnut would provide minimal societal benefits and does not warrant the costs to producers and EPA. Unlike other FIFRA exemptions, the requested exemption in this case will not require the public to forego the benefits of the full FIFRA registration. In this case, the principal oversight aims of FIFRA will be accomplished by the registration and tolerance exemption processes pending before the Agency. Avoiding unnecessary regulation benefits both producers and the Agency. Even after the exemption, the risk of any unknown, unexpected consequences is mitigated both by the producers' continuing duty to report any adverse effects associated with the exempt pesticide, and by EPA's clear legal authority to act in the immediate and longer term to regulate even exempt, unregistered pesticides, to the extent necessary to prevent unreasonable risk.

The Administrator's decision to grant this petition will be supported by extensive review and scientific and policy consensus on the safety of the tree and the practical impacts of its restoration performed by other federal agencies (i.e., USDA-APHIS and FDA) charged with evaluating the risks of transgenic plants within their jurisdiction. Their respective decisions will reflect the results of numerous opportunities for public comment on their respective assessments and tentative conclusions. Consensus among these agencies is expected to be achieved through interagency consultation under the federal Coordinated Framework for the Regulation of Biotechnology, most recently updated and strengthened earlier this year by Executive Order 14081, Advancing Biotechnology and Biomanufacturing Innovation for a Sustainable, Safe, and Secure American Bioeconomy (Sept. 12, 2022).

- First, EPA's Office of Pesticide Programs (OPP) is evaluating both an application by ESF to register the OxO gene in the Darling 58 American chestnut as a plant-incorporated protectant (PIP) under FIFRA section 3, and a petition under section 408 of the Food, Drug and Cosmetic Act for an exemption from the requirement of a tolerance for pesticide residues in food. These were submitted via the IR-4 Project, which was established by the USDA to facilitate regulatory approval of sustainable pest management technology for specialty uses to promote public wellbeing. To grant the registration and the tolerance exemption, EPA will need to conclude that addition of the OxO gene will not cause unreasonable adverse effects on human health or the environment, and that there is reasonable certainty that no harm will result from OxO residues in food (*e.g.*, chestnuts). 87 Fed. Reg. 16,470 (Mar. 23, 2022). The ESF pesticide registration application and supporting data previously or hereafter submitted in connection with the Darling 58 American chestnut and petition for tolerance exemption are incorporated herein by reference.
- Second, USDA-APHIS currently is reviewing ESF's petition for nonregulated status of the Darling 58 tree under the Plant Protection Act, 7 U.S.C. § 7701 *et seq*. To deregulate the tree, USDA-APHIS must first conclude that the plant is unlikely to pose an increased plant pest risk compared to the wild-type tree. APHIS recently completed and published its draft Plant Pest Risk Assessment (PPRA; Document ID APHIS-2020-0030-8290) and the associated draft environmental impact statement (EIS). The EIS contains APHIS'

assessment of the environmental impacts that may result from deregulating the tree, including the potential environmental impacts to managed natural and non-agricultural lands, the physical environment, biological resources, human health, socioeconomics, federally listed threatened or endangered species, and cultural or historic resources See, *e.g.* 87 Fed. Reg. 67,861 (Nov. 10, 2022) and Document ID APHIS-2020-0030-8289 (USDA-APHIS 2022). APHIS has tentatively concluded that "…the impacts of a determination of nonregulated status for Darling 58 American chestnut are unlikely to be adverse." (USDA-APHIS 2022 at 4.45).

- Third, the U.S. Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition is evaluating a dossier on Darling 58 chestnuts submitted by ESF under FDA's longstanding Plant Biotechnology Consultation Program, which provides a rigorous safety evaluation based on the objective characteristics of the food under consideration. (Biotechnology Notification File No. BNF185).

The record and decisions in these related proceedings will provide an adequate basis for decision on most aspects of this Petition. Petitioners ask the Administrator to consider this Petition in parallel with decisions in these related proceedings and to take official notice of the public record and decisions in those cases when and as available.

IV. BACKGROUND ON THE AMERICAN CHESTNUT AND THE BOLD UNDERTAKING TO RESTORE ITS PLACE IN FOREST ECOSYSTEMS OF THE EASTERN U.S.

A. The American Chestnut and the Problem of Chestnut Blight

The American chestnut (*Castanea dentata*) is a large, deciduous tree native to eastern North America. Before the introduction of chestnut blight, the American chestnut had a range that extended across eastern North America from Mississippi to Maine, including nearly every state east of the Mississippi River as well as southern Ontario, and was found at elevations from sea level to over 5000 ft. In many areas, especially on mountain slopes in the Appalachian range, American chestnut was a dominant forest tree and a keystone species.

Figure 1. Natural Range of American chestnut (Jacobs 2007).

American chestnut provided a consistent nut crop that was consumed by numerous mammals, birds, and insects. The nuts were important both ecologically and agriculturally, as they were also consumed by people and livestock. Chestnut wood and leaf litter influenced ecosystem structure and function in areas where it was a dominant tree (Ellison *et al.,* 2005).

In the first decade of the twentieth century, a non-native fungal canker disease was discovered in the Bronx Zoological Park, New York, which was disfiguring and quickly killing American chestnut trees (Merkel, 1905). In the decades following its discovery, the blight would spread throughout the chestnut range of North America and kill approximately 3½-4 billion trees (over 99.99% of the mature population). Early attempts were made to control the spread of the disease, including quarantine and destruction of diseased trees. These were generally ineffective (Rigling and Prospero, 2018), likely because of the fungus' ability to produce prolific spores which spread by animals and the wind, as well as its ability to reproduce as a saprophyte on other tree species and on dead American chestnut stems (Prospero *et al.,* 2006). These factors, in conjunction with American chestnut's extreme susceptibility to infection, overwhelmed all attempts at containment.

The blight fungus infects the stem via wounds or cracks in the bark. It has been shown that the pathogen kills living tissue primarily by secreting a toxin called oxalic acid (McCarroll and Thor, 1978). Once the fungal spores reach the inner bark, they produce hair-like mycelia, which spread in a radial pattern through the cambium layers and even into the sapwood. These mycelia spread at a rate of 5-10 inches per year. This disease produces red, orange, or yellow, fast-growing, sunken cankers on the tree's trunk, limbs, and sprouts. Eventually, these cankers will penetrate deeper into the trunk, encircle the entire trunk, often splitting or loosening the bark. These cankers will eventually girdle the trunk or the sprout. This girdling stops the transport of water and nutrients between the roots and the leaves, eventually starving and killing the above-ground portion of the tree. *See* MRIDs 51384801 and 51384802 for additional information on the biology of the plant and the blight fungus. Other tree species, mostly oak, maple and hickory, have filled the space left by dying chestnut trees and American chestnut is now considered functionally extinct in modern forests (Ellison *et al.,* 2005).

No wild-type American chestnut tree with documented blight tolerance has been found, but American chestnut has avoided extinction in the wild through its ability to sprout new shoots from the collar of diseased trees (Paillet, 2002). While the population has been reduced drastically, millions of (mostly immature) trees remain in the wild and in orchards (Fitzsimmons, 2017), which should allow re-establishment of a viable, genetically diverse population if a heritable blight tolerance trait can be introduced. *See* MRIDs 51384806 and 51874207 for more information on restoration considerations and potential distribution strategies.

B. The Blight-Tolerant Darling 58 Chestnut Tree

Petitioner ESF, with significant support from TACF and others, has developed "Darling 58," an American chestnut with expression of the wheat gene for oxalate oxidase, which degrades oxalic acid and protects the tree from damage caused by chestnut blight. The OxO gene sequence used in the Darling 58 was derived from wheat (*Triticum aestivum)* (Dratewka-Kos *et al.,* 1989), but OxO enzymes are naturally present in a wide variety of food products and wild plants. (MRIDs 51384802 – 51384804). The OxO gene is accompanied by NPTII from *E. coli* as a selectable marker, which has been previously granted a tolerance exemption by EPA (40 C.F.R. § 174.521). The Darling 58 was developed using the proven *Agrobacterium*-mediated transformation technique. (MRIDs 51384802; 51874201). Complete product chemistry is provided for the record (MRIDs 51384801 – 51384803; 51874201 – 51874203).

The Darling 58 has demonstrated stable, reliable integration of a single copy of the OxO gene into a non-coding region of the American chestnut genome, without the addition of extraneous *Agrobacterium* sequences. Darling 58 tissues have been characterized using quantitative PCR, genome walking, genome sequencing, reverse transcription qPCR (RT-qPCR) to quantify mRNA transcript levels, and by quantitative enzyme assays which detect byproducts of the oxalic acid degradation reaction (MRID 51874201 – 51874203).

The OxO enzyme expressed by the Darling 58 American chestnut catalyzes the degradation of oxalic acid into carbon dioxide and hydrogen peroxide, which allows the Darling 58 American chestnut to protect its living tissues from damage by oxalic acid exuded by the blight fungus. Degradation of oxalic acid reduces necrosis of living tree tissues at the margins of cankers. This mechanism is not lethal to the fungus or inhibitory to its replication; it continues to live as a saprophyte on Darling 58 American chestnut trees as it does on Chinese chestnuts and many other tolerant tree species (Nash and Stambaugh, 1987; Baird, 1991). Thus, restoration using transgenic American chestnuts is not expected to drive evolutionary changes in the blight fungus that would overcome the tree's new tolerance mechanism. Since OxO does not exert a strong selective pressure on the fungal organism, the protection afforded by the transgene to the American chestnut is expected to be durable over generations. Efficacy of Darling 58 trees tolerating blight infections has been demonstrated in both leaf and stem inoculation assays (MRID 51384802), which compare the response of Darling 58 to wild-type American chestnut and Chinese chestnut controls.

The Darling 58 American chestnut tree is a long-lived forest tree intended for use in environmental restoration, not a patented agricultural crop plant intended for managed commercial harvest. It is intended to outcross with wild relatives, which would allow the transgene to introgress into wild populations. It was intentionally not developed in a homozygous transgenic state, which will allow enhanced diversity via outcrossing and continual production of fully wild-type offspring. American chestnut can hybridize with native chinquapins (*e.g.*, *C. pumila* and *C. ozarkensis*), which, like American chestnut, were suppressed by blight but continue to persist within their former range in the presence of blight. The natural production and proliferation of such hybrids would be restricted by differential habitat types and the rarity of mature wild trees of both species, as well as varying flowering times among species. If they were to occur, Petitioners would not expect such hybrids to possess any greater invasive tendencies than pre-blight American chestnut-chinquapin hybrids.

It is anticipated that it will take decades or longer for the American chestnut to proliferate, and then only with significant intentional human intervention. *See, e.g.*, USDA-APHIS 2022 at 4-18. The American chestnut is relatively slow to spread, not weedy, easy to identify, slow to flower, requires two flowering individuals to make viable seed, and take years to produce seeds. They are easy to exclude from an area if someone desires to do so. *See* MRID 51874207 for additional discussion of estimated timelines and proposed distribution plans.

Petitioners plan to reintroduce the tree to native forests by first crossing it with regionally sourced individuals in order to incorporate appropriate genetic diversity from surviving American chestnuts from various parts of their native range. Careful attention will be given to predicted future habitat suitability (Barnes and Delborne, 2019). The products of this breeding program may ultimately be introduced to forests on a larger scale (in coordination with public and private forest land managers), with the aim of establishing a self-sustaining, diverse, resilient, blight-tolerant population of American chestnut trees. Petitioners intend that trees and pollen also will be made available to the public for restoration and demonstration purposes on a small-scale once regulatory approvals are finalized.

V. RESTORATION OF THE AMERICAN CHESTNUT TO ITS NATURAL RANGE WOULD BRING CERTAIN AND SIGNIFICANT ENVIRONMENTAL, CULTURAL AND ECONOMIC BENEFITS

The principal benefits of restoring the American chestnut to its natural range are primarily ecological and cultural. There is the potential for long term economic benefits for timber industries at a time when a sufficient chestnut density has been achieved, but that possibility is only many decades in the future and, like other hardwood trees, is unlikely to be achieved through monocrop plantations. Similarly, while American chestnuts can provide valuable and nutritious human foodstuffs in the form of nuts, the diminutive size and other characteristics of the American chestnut compared to Chinese and hybrid chestnuts makes commercial orchards established for this purpose to be highly unlikely (USDA-APHIS 2022 section 4.7).

A. Ecological Benefits of Restoring the American Chestnut to its Natural Range

We agree with APHIS that biodiversity resulting from effective forest conservation and restoration plantings would have direct benefits to ecosystem services, which otherwise would require costly management practices (USDA-APHIS 2022). Decades of intentional plantings and maintenance of blight-tolerant American chestnuts may allow them to thrive and eventually partially displace other tree species that have taken their place over the past century. Effective chestnut restoration also would favor many native wildlife species that once relied on the nutritious and consistent nut crop, such as white-tailed deer, cottontail rabbits, many rodents, wild turkeys, and ruffed grouse, potentially resulting in increases in broader biodiversity (Hill, 1994; Dalgleish and Swihart, 2012). APHIS also anticipated potential impacts (generally likely to be beneficial) from tree restoration associated with air quality, surface water and groundwater, wildlife habitats, and carbon sequestration (USDA-APHIS 2022 section 4.9).

If blight-tolerant American chestnut were to become established as an important canopy tree, it would begin to influence ecosystem structure and function in these areas, as it did prior to the blight (Paillet, 2002). An increase in reliable hard mast production would likely result from the reintroduction of American chestnut, which in turn could result in population increases of species that feed on chestnut, including many mammals and birds as noted above. This increase would be most pronounced during years of low seed production by other masting species (such as oaks), resulting in less fluctuation in consumer species (Dalgleish and Swihart, 2012). The consistency of chestnut mast crops is of particular importance, since strong mast pulses generally lead to instability or chaotic ecosystem effects (Kelly *et al.,* 2008). Higher rodent populations could increase the pest potential of these species, and human-wildlife interactions could increase with greater large mammal populations. Population increases of small animals could also increase predator populations until a balance is reached.

One specific example of a small mammal that might benefit from American chestnut reintroduction is the threatened Allegheny woodrat, whose population declines were likely exacerbated by the loss of chestnuts to blight (Balcom and Yahner, 1996; Monty and Feldhamer, 2002). Chestnuts were an important food source for woodrats prior to the introduction of blight, so in conjunction with suitable habitat management, chestnut reintroduction could help protect remaining populations of these unique rodents (Wright and Kirkland, 2000). There have also been reports of native insects such as Chestnut moths that declined after blight infestations, and may be revived or rediscovered in parallel with chestnut restoration (Hill, 1994).

Chestnut trees grow quickly, and their wood is slow to decay due to high tannin concentrations (Wang *et al.,* 2013). These features may provide rapid sequestration of carbon and nutrients, increasing long term carbon storage, especially when used in afforestation (Ellison *et al.,* 2005), though the magnitude of this effect compared to other hardwood species may be minor (Gustafson *et al.,* 2017).

Chestnut leaf litter may positively impact terrestrial and aquatic ecology. There is some evidence that chestnut leaf litter has allelopathic effects, suppressing competitors including eastern hemlock and rhododendron (Vandermast *et al.,* 2002), but allelopathy was not observed in a recent study on germination of other native seeds (Newhouse *et al.,* 2018). Chestnut leaf litter decomposes more rapidly in the first year than oak or cherry leaf litter, and soils with chestnut leaf litter were shown to have lower N leaching rates and greater dissolved organic carbon than soils with cherry or oak leaf litter (Schwaner and Kelly, 2019). These differences represent a potential for increased storage of carbon in surface soil of forests with re-introduced American chestnut as the microbial community accumulates biomass in an N-limiting environment.

Chestnut leaf litter is of higher nutritional value for aquatic macroinvertebrates than oak, so the replacement of oak by chestnut may increase macroinvertebrate activity, with potential consequences at population, community, and ecosystem levels (Smock and MacGregor, 1988). Additionally, slow-decaying chestnut wood may increase stream channel complexity as it replaces faster-decaying species, providing additional habitat for fish and invertebrates (Ellison *et al.,* 2005).

American chestnut can hybridize with native chinquapins, which, like American chestnut, continue to persist within their former range in the presence of blight. We would not expect such hybrids to possess any greater invasive tendencies than pre-blight American chestnut-chinquapin hybrids, which were known to occur (Shaw *et al.,* 2012). However, a hybrid inheriting the trait of blight tolerance could potentially have a competitive advantage over blight-susceptible chinquapins. While blight-tolerant American chestnut would be sexually compatible with Asian and European species, and may be intentionally bred with these species by horticulturalists, we would not expect such hybrids to possess weedy characteristics, based on the lack of reported weediness of any non-native or hybrid *Castanea* in North America to date.

American chestnuts can potentially offer unique ecological benefits in coal mine reclamation efforts, where their tolerance of acidic well-drained soils and lack of canopy competition may be beneficial to their early-successional establishment (Skousen *et al.,* 2013; Bauman *et al.,* 2014). This could in turn help broader reclamation efforts on these or other severely degraded sites.

B. Cultural Benefits of Restoring the American Chestnut to its Natural Range

The American chestnut had a significant impact on our history and culture, and is immortalized in song, stories, and place names. Prior to the chestnut blight, the chestnuts themselves were a subsistence crop to rural Americans and Indigenous peoples, who consumed nuts directly, fed them to livestock, or traded them to merchants, who in turn would sell them as a seasonal delicacy in eastern U.S. cities (Davis 2005). Chestnut leaves were once sold as crude medicine, and recent studies may lead to modern pharmacology products (Silva *et al*. 2020). Successful American chestnut restoration will have beneficial impacts on forest biodiversity, native wildlife populations (including popular game species), availability of a wild agroforestry crop, and a nutritious alternative or additive for livestock feed (USDA-APHIS 2022). The cultural importance of the American chestnut, the significance of its loss, and the benefit of its return are vividly portrayed in, among other places, in a 2018 video presentation by the Templeton World Charity Foundation (Templeton, 2018). *See generally* the Additional Resources cited in the Appendix.

When the blight was discovered at the Bronx Zoo in New York City in 1904, it commenced a conservation and rescue effort like no other, which continues to this day despite the tree being virtually eliminated as a canopy tree and functionally extinct since the 1950s. Hundreds of academic researchers, horticulturalists, and geneticists, combined with governmental agencies such as the Pennsylvania Blight Commission, the Connecticut Agricultural Research Station, and the U.S. Forest Service, have worked tirelessly to find a solution to save the devastation of this keystone species. For decades, different techniques and experiments were launched with little or no success. Many researchers from the 1920s to the 1960s devoted their life's work to this species, only to pass on before a solution was found. These well-intentioned public efforts were mostly abandoned until The American Chestnut Foundation formed in 1983 and ESF's American Chestnut Research and Restoration Project in 1990. These efforts resurrected the mission to save the American chestnut.

New hope is in sight to develop a blight-resistant American chestnut in our lifetimes, ready for large scale restoration that will be transformational in its benefits to forest health and tree disease resistance. This is possible due to innovations such as blight tolerance developed through biotechnology at ESF combined with TACF's traditional tree breeding program to ensure regional adaptation and genetic diversity of restoration-ready trees. These programs would not be possible without the enthusiastic support of the public combined with private philanthropy. ESF and TACF field inquiries daily from chestnut enthusiasts asking when the blight-resistant chestnut will be available to the public. Efforts to restore the American chestnut have captured the imagination of people across the United States, hoping to be part of this grand rescue mission. The reintroduction and effective de-extinction of the American chestnut is unparalleled in conservation history.

There is tremendous public interest in support of American chestnut restoration. One place that this can be seen is in the outpouring of public support reflected in the positive comments submitted to USDA-APHIS at different stages during its review of ESF's petition for nonregulated status for the Darling 58 tree under the Plant Protection Act. The testimonies of support and connection to family and place are often incredibly eloquent and moving. The stories' passion, optimism, and hope for the future expressed in these comments capture both what has been lost with the functional extinction of the tree, and what is to be gained by its restoration. In addition to comments from the general public, a large number of professional scientists, foresters, and naturalists also have submitted comments supporting restoration of the tree. And more than 100 agencies and scientific and conservation organizations have submitted comments supporting deregulation of the tree, including the Sierra Club and The Nature Conservancy. *See, e.g.,* APHIS-2020-0030-4145, and APHIS-2020-0030-3813 and -11539. Also noteworthy is the quality of the relationship with Federal and state agencies, particularly with the U.S. Forest Service.

With respect to the cultural benefit of restoring the American chestnut, several common themes are apparent in the public comments:

1. Many commenters note a strong connection to both past and future family members, and that they wish to see deregulation of Darling 58 to ensure a legacy or dream can come true for loved ones already lost or yet to come. *See, e.g.,* APHIS-2020-0030-11087, -11726, -9161, and -12758.

2. Many hikers, hunters, and other lovers of the natural world note their connection to the forests in which they regularly recreate, seeing the infected stems of dead and dying American chestnuts, and wanting to see them once again a vibrant part of the forest. *See, e.g.,* APHIS-2020-0030-11928 and -9759.

3. Because humans spread chestnut blight more rapidly than otherwise would have happened, humans need to address the problem with all reasonable means. *See, e.g.,* APHIS-2020-0030-11459 and -2718.

4. Many embrace the cause as a rare positive environmental story for the future, noting the technology should be used to save other species. *See, e.g.,* APHIS-2020-0030-8623, -11200, -12397, and -13005.

5. Finally, and analogous to the daily calls received by staff persons at The American Chestnut Foundation, several hundred commenters note that they have land on which they want to plant Darling 58 trees. *See, e.g.,* APHIS-2020-0030-8594 and -12605.

As noted, these comments were directed to USDA-APHIS' action under the Plant Protection Act, but they speak to the broader cultural value from the restoration of the American chestnut. The strong public interest also is reflected in the attention and support reflected in the mainstream press. *See generally* the Additional Resources cited in the Appendix.

C. Economic Benefits of Restoring the American Chestnut to its Natural Range

The American chestnut is a fast-growing hardwood species that produces an abundant nut crop, straight-grained and rot-resistant timber, and historically provided a source of tannins for the leather industry (Wang *et al*. 2013). As recently found by USDA-APHIS, prior to the blight, chestnut trees were a common source of timber, poles, shingles, trim, and firewood in the eastern U.S. where they were native (USDA-APHIS 2022). Their consistent nut crop was historically valuable for rural families to forage and sell, or to let livestock graze in wooded areas. Today, chestnuts are still considered a nutritious food product, and are among the most prevalent tree nuts sold worldwide. However, most of the global production comes from China, and even the burgeoning chestnut industry in the U.S. is primarily focused on growing Asian or hybrid chestnut trees due to their optimal agronomic traits (Davison *et al*. 2021). If transgenic blight-tolerant American chestnuts were widely planted for restoration and allowed to mature, they would be expected to have essentially the same characteristics as wild American chestnuts did before blight: straight timber form, tall growth, and prolific nut production. This would eventually allow for a useful and valuable timber product, but nut production for commercial sale will likely continue to come from elite hybrid varieties (USDA-APHIS 2022). In the immediate future, economic benefits from reintroducing blight-tolerant American chestnuts likely will be related to planting and restoration activities, by private landowners with cultural and historical interests, and by others seeking to meet incentivized restoration and carbon sequestration goals by planting unique native hardwood tree seedlings.

ESF, TACF and its many non-profit, academic, and agency partners are gearing up for large scale reintroduction of disease-tolerant American chestnuts. This effort to produce and plant millions of seedlings to the forest will require an investment of time and resources on a scale not seen since the Civilian Conservation Corps. This audacious goal is a de-extinction vision never before attempted. The investment in people, jobs, and facilities will ensure that the necessary infrastructure will be available not only for the rescue of specific forest trees, but also the development of diverse forests for ecosystem preservation, carbon sequestration, and restoration of disturbed areas like abandoned mine lands, which also require extensive rehabilitation with diverse tree species.

VI. THE ADMINISTRATOR HAS THE AUTHORITY TO EXEMPT OXO IN THE AMERICAN CHESTNUT FROM FIFRA

FIFRA generally requires that "pesticides" must be registered by EPA before they may be distributed or sold. FIFRA defines a pesticide as any "substance" "intended for preventing, destroying, repelling, or mitigating any pest[.]" FIFRA §2(u); 40 C.F.R. §152.3. The OxO enzyme is not intended to directly kill or mitigate the chestnut blight fungus (*Cryphonectria parasitica*) itself. Rather, it only prevents some of the damage to tree tissues that would otherwise be caused by the chestnut blight fungus. The OxO expressed by the transformed tree neutralizes oxalic acid released by the fungus to minimize (lessen) the damage to the tree. This permits the tree to tolerate the fungus, but still allows the fungus to survive and reproduce, so the two can coexist. Nevertheless, EPA deems the OxO in the American chestnut to be a plant-incorporated-pesticide (PIP) due to the "mitigation" of the effects of the blight fungus. As noted, ESF's application to register the PIP is pending.

Although in EPA's view OxO technically may qualify as a "pesticide," EPA is not required to regulate it where not warranted under the circumstances. In section 25(b)(2) of FIFRA, 7 U.S.C. § 136w(b)(2), Congress authorized the Administrator, by rule, to exempt a "pesticide" from regulation when, *inter alia*, the Administrator determines the pesticide is "of a character which is unnecessary to be subject to [FIFRA] in order to carry out the purposes of the [Act]." Using this authority, EPA has determined not to regulate a number of specific substances and categories of substances that otherwise meet the definition of a "pesticide" in some circumstances. *See* 40 C.F.R. § 152.25 (*e.g.*, treated articles, pheromones, preservatives for biological specimens, foods, natural cedar, certain defined minimum risk pesticides); 40 C.F.R. § 174.25 (PIPs from sexually compatible plants). EPA interprets FIFRA section 25(b) to authorize the Agency to exempt a pesticide or category of pesticides where the Agency determines:

1. The pesticide poses a low probability of risk to the environment, and
2. The pesticide is not likely to cause unreasonable adverse effects to the environment even in the absence of regulatory oversight under FIFRA.

See, e.g., 85 Fed. Reg. 64,313 (Oct. 9, 2020). In evaluating the first criterion, EPA considers the extent of the potential risks to the environment arising from use of the pesticide, including dietary and non-dietary effects on humans and other animals, plants, water, air, and land. *See e.g.*, 72 Fed. Reg. 19,590, -92 (Apr. 18, 2007). EPA cannot grant an exemption under FIFRA unless the pesticide poses a low probability of risk in these circumstances. In evaluating the second criterion, EPA balances potential risks to human health and the environment from unregulated use of the pesticide against the potential benefits associated with its use. In balancing risks and benefits, EPA considers the economic, social, and environmental costs and benefits of the use of the pesticide in relation to the societal benefits to be gained by imposing the full degree of oversight associated with FIFRA registration. *See, e.g.*, 85 Fed. Reg. 64,308, -13, -24 (Oct. 9, 2020).

VII. OXO IN THE AMERICAN CHESTNUT POSES A LOW PROBABILITY OF RISK TO HUMAN HEALTH AND THE ENVIRONMENT

In evaluating whether the use of a pesticide poses a low probability of risk to the environment, EPA considers the extent of the potential risks caused by use of the pesticide to the environment, including humans and other animals, plants, water, air, and land. 72 Fed. Reg. 19,590, -92 (Apr. 18, 2007). EPA cannot grant an exemption under FIFRA unless the pesticide poses a low probability of risk to the environment.

The OxO gene does not pose risk to the environment, including humans and other animals, plants, water, air, and land. Below, we discuss specific risk considerations, including concerns regarding genetic engineering. Many public concerns about genetically engineered chestnuts are either not specific to genetic engineering (*i.e.*, would also apply to backcross hybrid chestnuts), or are not specific to chestnuts (*i.e.*, the ultimate concern is actually about application of genetic engineering to other tree species unrelated to chestnuts or restoration efforts). Given the ideological nature of some such claims, some skepticism will not likely be satisfactorily addressed with data. As mentioned elsewhere, vocal ideological opposition has been a small minority voice in conversations about genetic engineering for chestnut restoration. This was reflected in the first public comment period for the APHIS Petition for Nonregulated Status for Darling 58 (Docket APHIS-2020-0030), in which the majority of unique responses were supportive (see section V.B above). Noteworthy examples include multiple supportive comments from The Nature Conservancy (Comment IDs APHIS-2020-0030-3813 and -11539), the Environmental Defense Fund (APHIS-2020-0030-4039), and a letter from the U.S. Forest Service (MRID 51874207). A National Research Council report (National Research Council, 2015) reminds us that "…the presence of small groups of passionate stakeholders does not suggest that the wider public is in a state of division about GMOs." More specific concerns (many of which are refuted in other MRID volumes and the USDA-APHIS Petition) include an assumed lack of genetic diversity, potential nontarget effects, and unwanted introgression with wild populations.

Blight tolerance achieved by the very limited (one gene) alteration to the American chestnut genome creates far less uncertainty than alternative, traditional methods. Both hybrid breeding and mutagenesis result in vastly more genomic changes than genetic engineering (Schnell *et al.,* 2015; Anderson *et al.,* 2016) – hundreds or thousands of new genes incorporated into the organism rather than one or two, as well as substantial structural rearrangements to genes and whole chromosomes. Even *Agrobacterium* transformation of plant genomes with bacterial DNA has been frequently observed in a variety of wild plants (Matveeva, 2021), so this process happens in nature and is not inherently more risky than "traditional" methods of plant breeding. Other concerns about genetic engineering may be conflated with agricultural traits like herbicide tolerance or insect resistance, or with motivations of for-profit agricultural companies, none of which are directly relevant to transgenic American chestnuts for restoration use.

A. Low Probability of Human Health Risk

Evaluation of human health risk considers both dietary risk and non-dietary risks such as those resulting from occupational or residential exposure to the pesticide.

1. Human Dietary Risk

Although commercial development of American chestnuts as a food is unlikely (*see* section V.C above and MRIDs 51384817 and 51874207), dietary exposure (nuts) is the most likely route of human exposure to OxO. The benchmark for evaluating human dietary risk is the Federal Food Drug and Cosmetic Act's (FFDCA) section 408 standard for setting pesticide residue tolerances and exemptions from tolerances. Under that standard, EPA must determine that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." 72 Fed. Reg. 19,590, -92; 21 U.S.C. § 346a(c)(2)(A)(ii).

The OxO enzyme is common in human diets and does not pose dietary risk to humans. OxO and similar enzymes are ubiquitous among human food and livestock dietary components, most monocot crops such as wheat, maize, rice, barley, sorghum, banana, and many dicot crops including strawberry, sunflower, beet, apricot, and peanut. It also is found in many species of wild plants routinely consumed by wildlife, including, for example, goatgrass, spiny amaranth, ramie, bougainvillea, stiff brome, rubber bush, white rot fungus, insulin plant, azaleas, and perennial ryegrass. Nutritional analysis of Darling 58 chestnuts confirmed that there are no substantial nutritional or compositional differences between wild-type and transgenic nuts. Analysis of the OxO enzyme indicates a lack of allergenicity or toxicity (MRIDs 51384804 and 51874205).

As noted, the Agency is currently reviewing a standard tolerance exemption petition for the OxO gene and related inert ingredients. EPA's determination of that petition will resolve the question for purposes of determining this aspect of the exemption petition.

2. Human Occupational and Residential Risk

Although the theoretical potential for human or occupational dermal or inhalation exposure exists, Petitioners expect such exposure to be negligible because the OxO is contained within the plant cells. In some cases, OxO residues may be present in other exudates from the plant or processed plant parts thus may present some limited opportunity for dermal exposure to persons coming physically into contact with the plant or raw agricultural food from the plant, but this would present a similar hazard to equivalent exposure to other unregulated plants containing OxO, which are much more common than exposure to chestnuts. As described in MRIDs 51384804 and 51874205, the lack of documented risks from exposure to other OxO-containing plant products, and the lack of risks observed across hundreds of researchers who have worked with transgenic chestnut trees so far, confirms that any risk to humans from chestnuts containing OxO would not be greater than the risk from other unregulated products.

B. Low Probability of Environmental and Ecosystems Risks

1. Limited Environmental Exposure

The potential for exposure to OxO is typically lower than for other types of pesticides because OxO is produced within the living plant and used in situ in the plant. Physiological constraints limit the amount of pesticidal substance produced by the plant. Accordingly, the routes by which humans and other organisms may be exposed to OxO are more limited that for non-PIP (3:1) pesticides (*See* MRID 51384804). In addition, OxO, like other PIPs, is inherently biodegradable and subject to biodegradation and decay in nature (*See* MRID 51384805), and reduction to its constituent elements through animal and human digestion by other living organisms. Because they are readily degraded, PIPs do not bioconcentrate in the tissues of living organisms or persist in the environment. *See* 85 Fed. Reg. at 64,321. Given these characteristics, the potential for new environmental exposures to OxO to occur beyond dietary exposure will be limited.

2. No Adverse Effects on Non-Target Avian, Mammalian, Freshwater Fish Species

OxO is naturally found in many wild plants and microbes consumed by wild animals, including in perennial ryegrass, goatgrass, azalea, castor bean, spiny amaranth, ramie, bougainvillea, stiff brome, rubber bush, white rot fungus, insulin plant, goose neck moss, endophytic bacterium, split-gill mushroom, dermatophytic fungus, wild einkorn wheat, narrowleaf cattail, and multiple mosses (*see* MRID 51384805 and Powell *et al.* 2019). The ubiquitous nature of oxalate oxidase and related enzymes implies that avian, wild mammalian and freshwater fish species are already widely exposed to OxO in the environment, not only from ingested plant material, but also potentially from fungal spores, mosses used as nesting materials, and omnipresent bacteria. Due to its specificity, oxalate oxidase will be enzymatically active only in the presence of oxalic acid, most likely produced by *Cryphonectria parasitica* or other pathogenic fungi, and not produced by animals. OxO may be present in plant tissues but will have no effect on animals, and if ingested (such as in chestnut seed tissue) it will be digested as dietary protein by hydrochloric acid and proteases in animal stomachs, just as it is from cereal grains and other dietary sources. *See* MRID 51384805 and Goldspiel *et al*. (2019) for additional information on exposure of OxO and lack of toxicity to avian, mammalian, and freshwater fish organisms.

3. No Adverse Effects on Non-Target Freshwater Invertebrate Species

As described in MRID 51384805, exposure of freshwater invertebrates to OxO will be limited. Tests on caddisfly larvae reared on transgenic chestnut leaves showed no detrimental effects of OxO to these uniquely sensitive freshwater invertebrates (MRID 51384805 at 28-29).

4. <u>No Adverse Effects on Non-Target Insects (Including Pollinators)</u>

Multiple experiments involving insects feeding on transgenic chestnut leaves and using pollen containing the OxO enzyme have been completed and reported to the Agency (*see, e.g.*, MRID 51384805). In all cases, there is no indication that OxO will be detrimental to non-target insects. Brown et al. (2020) found no differences in leaf consumption or insect survival after feeding on Darling 58 vs. non-transgenic control chestnut leaves. Likewise, a study on chestnut pollen use by bumble bees showed that OxO did not significantly affect survival, pollen usage, or reproduction in these pollinators (Newhouse et al. 2021).

5. <u>No Adverse Effects on Non-Target Plants</u>

The OxO gene in the American chestnut does not pose a plant pest risk. This is shown by several studies described in MRID 51384805. Additionally, USDA-APHIS recently completed a Plant Pest Risk Assessment of this event and, subject to further public comment, has concluded that that the Darling 58 American chestnut expressing OxO is unlikely to pose a greater plant pest risk than its unmodified parent. (USDA-APHIS 2022a). APHIS was satisfied that the current data is sufficient to establish, *inter alia*:

- No plant pest risk was identified from the transformation process.
- No increase in plant pest risk was identified from expression of the inserted genetic material, the new OxO or NPTII proteins, or changes in metabolism or composition.
- Exposure to and/or consumption of Darling 58 American chestnut is unlikely to have any adverse impacts on organisms beneficial to agriculture based on USDA-APHIS' analysis of studies on Darling 58 American chestnut food and feed safety, nutrient and anti-nutrient composition, levels of OxO and NPTII in tissues, and environmental interactions with beneficial arthropods, and pollen characteristics.
- Disease and pest incidence and/or damage, other than reductions in chestnut blight from the target organism *Cryphonectria parasitica*, were not observed to be significantly different or atypical in OxO-expressing American chestnut compared to unmodified controls in field trials. No plant pest effects are expected on these or other forest or agricultural products and no impacts are expected to USDA pest control programs.
- American chestnut with OxO is unlikely to become more of a weed or volunteer problem than wild-type American chestnut based on its observed agronomic characteristics, the low weediness potential of American chestnut, and current management practices available to control spread of chestnuts. American chestnut volunteers (transgenic or wild-type) can be controlled with all currently available weed control methods, should a landowner wish to remove any type of chestnut from their property.
- OxO-expressing American chestnut is not expected to increase the weed risk potential of other American chestnut, or other *Castanea* species with which it can interbreed.
- The genetic modification in Darling 58 American chestnut is not expected to increase its potential for gene flow, hybridization, and/or introgression to sexually compatible

taxa, nor is it likely to increase their weediness potential in the event that such species were to be introduced.

- Horizontal gene transfer of the new genetic material inserted into Darling 58 American chestnut to other organisms is highly unlikely and is not expected to lead directly or indirectly to disease, damage, injury, or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

6. Horizontal Gene Transfer to Soil Organisms Unlikely

USDA-APHIS rigorously evaluated the likelihood and potential implications of horizontal gene transfer in their draft Plant Pest Risk Assessment (USDA-APHIS 2022). It concluded that "horizontal transfer from and expression of DNA from a plant species to bacterial, fungal, or invertebrate species is unlikely to occur…" (USDA-APHIS 2022 page 18). As described above, OxO and similar genes are already naturally present in many microbes and wild plants (Powell *et al*. 2019), so its presence in the environment and potential transfer is not unique to transgenic chestnuts.

7. Gene Flow and Lack of Invasiveness

The only logical means by which transgenes from American chestnuts could spread to related species is through inheritance by viable offspring from successful pollination with at least one transgenic parent (*see* MRID 51384807). American chestnut is capable of hybridizing with other members of the genus *Castanea*, either through controlled pollination or open pollination, though differential timing of flower development may represent one possible barrier to open pollination. Interspecific *Castanea* hybrids are sometimes male-sterile (unable to produce viable pollen) but are capable of setting fruit if female flowers are pollinated by a compatible donor (Anagnostakis, 2012; Sisco *et al*., 2014).

European, Chinese, and Japanese chestnuts have been cultivated for thousands of years for timber and nut production (Goodell, 1983). Intentional interspecific hybridization has been performed for more than a century in most chestnut growing regions worldwide (Pereira-Lorenzo *et al*., 2016). Hundreds of cultivars and interspecific hybrids have been named, many of which are commercially available from nurseries (Anagnostakis, 2012). European chestnut material was imported into the United States as early as 1773, and Japanese chestnuts were first imported in 1876 (Anagnostakis, 2012). Chinese chestnuts were imported as early as 1901, and both Seguin and Henry chestnuts followed soon thereafter (Galloway, 1926). Many of these introductions were conducted by the USDA for agricultural purposes, including blight-resistant breeding stock to create Asian-American hybrids. Interspecific crosses of American chestnut with Chinese chestnut (*C. mollissima x C. dentata* or the reverse) and with other Asian *Castanea* species are not universally successful (Jaynes, 1964), but fertile offspring are produced often enough to allow hybridization programs to be constructed with the intent of mitigating chestnut blight (Jacobs *et al*., 2013).

North American *Castanea* species appear to hybridize where their distributions overlap (Figure 1). Tucker (1975) observed an intergradation in features where Ozark chinquapins in the mountainous interior overlap with the Allegheny chinquapins on the coastal plain. In the central and southern Appalachians, some authors have reported plants with intermediate morphology between American chestnut and Allegheny chinquapin; this population has been described as the hybrid taxon *C. x neglecta* (Johnson, 1988). Other authors have described a taxon called *C. x alabamensis* (Elias, 1971), which may be another *C. dentata* x *C. pumila* hybrid, though it has also been considered an isolated population of *C. ozarkensis* (Johnson, 1988), or an entirely separate species (Ashe, 1925). More recent analyses of *C. x alabamensis* confirm it is morphologically and phylogenetically unique from *C. dentata* and not likely a hybrid, but leave its species status unresolved (Perkins *et al.*, 2019). The evolutionary history of North American *Castanea* species is still not fully understood and is "complicated by recent and past hybridization and incomplete lineage sorting" (Shaw *et al*., 2012).

Though many more years of research will be required to produce comprehensive data about interspecific hybridization of transgenic American chestnuts and compatible species, there is no reason to expect that pollen viability, fertilization rates, or any other aspect of sexual reproduction would differ between transgenic and non-transgenic American chestnut. Indeed, successful controlled pollination has been accomplished from Darling 58 pollen on flowers from *C. sativa*, *C. mollissima*, *C. ozarkensis*, *C. pumila*, and various hybrids of these species. Preliminary studies indicate that the OxO gene is inherited as expected, and that expression of OxO in hybrid offspring of these crosses is not substantially different from the range of expression found among offspring of the original *C. dentata* lines (Figure 2). Male sterility in hybrid offspring appears to be a result of interactions involving cytoplasmic (chloroplast or mitochondrial) genes in American chestnut (Sisco *et al*., 2014), so the presence of transgenes in the nuclear genome of Darling 58 should not change these interactions. Therefore, we expect that transgenic American chestnuts growing in close proximity to other *Castanea* species could produce viable hybrid offspring. Historically, American chestnut was reported to occasionally hybridize with chinquapin species where their ranges overlapped (Figure 1). Hybrid offspring inheriting the OxO gene would be expected to exhibit enhanced blight tolerance.

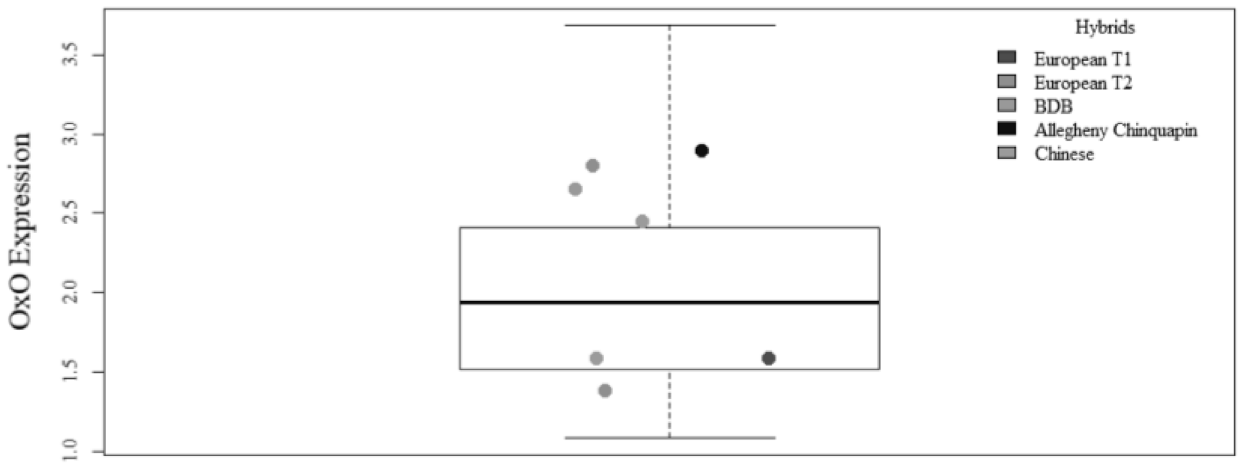

Figure 2. Box plot of relative OxO mRNA expression across 35 transgenic American chestnuts. Overlaid in colored dots is relative OxO mRNA expression of seven chestnut hybrids of five types (European x American T1 and T2, Bouche de Betizac x American, Allegheny chinquapin x American, and Chinese x American). Note that expression values for all hybrids fall within the range shown by full American transgenic chestnuts.

Given the expected slow rate of chestnut reproduction and spread, and limited effective pollination distances, such natural hybridization events would be rare. This is especially true while mature transgenic chestnuts and their offspring are uncommon, a time period of several decades to several centuries following the introduction of Darling 58 to the landscape. There are few peer-reviewed studies on the effective pollination distance for American chestnut but reported practical applications from chestnut growers and results from related species are informative. These reports include both wind and insect pollination because they examine viable nut production success, regardless of how the pollen is vectored. Jacobs *et al*. (2011) report that trees need to be within 100 meters for successful pollination. Rutter (1990) states that "trees only 100 feet [30 m] apart will experience reduced pollination success, and trees 1000 ft. [300 m] apart are essentially reproductively isolated from one another." These reports confirm a conclusion that unintentional transgene flow will be unlikely unless or until mature Darling 58 offspring become prevalent on the landscape.

Plant breeders may intentionally create hybrid chestnut varieties for agricultural or horticultural use, incorporating the OxO gene for blight tolerance. This also could be achieved through separate transformations directly into other *Castanea* backgrounds rather than by hybrid breeding. Hybridization could occur between transgenic American chestnut trees in orchards and nearby wild *Castanea* individuals, or between wild transgenic chestnut trees and *Castanea* trees in orchards. Depending on blight susceptibility and goals of land managers, occasional natural introgression of the trait into other chestnut species would not necessarily be met with opposition. We have not seen any detrimental effects on human health, non-target organisms, or nut quality in transgenic American chestnuts, so it is unlikely that the inclusion of OxO would have a detrimental effect on other *Castanea* species or hybrids, or other organisms in their respective environments. There is a possibility of a positive effect from enhanced blight tolerance on wild and naturalized populations of *Castanea* species that are susceptible to chestnut blight, which include the American chestnut, European chestnut, Allegheny chinquapin, and Ozark chinquapin.

8. <u>Low Pest Resistance Selection Pressure on *C. parasitica*</u>

A component of EPA's oversight historically for PIPs created through biotechnology is concerned with pest resistance selection pressure. Evolutionary selection pressure created by effective PIPs can result in pest resistance to a chemical and significantly undermining the efficacy of the PIP. There is low risk that *C. parasitica* will evolve to overcome the tolerance conferred by an oxalic acid tolerance trait, nor is there risk that the tolerance would break down or fail if deployed to the natural ecosystem. The continued presence of *C. parasitica* on OxO-expressing American chestnut trees has the unique benefit of reducing selective pressure for the fungus to overcome the action of the OxO enzyme. This unique tolerance mechanism, in which the tree continues to serve as a host for fungal colonization and reproduction, and the continued presence of non-transgenic trees in the landscape (including offspring from transgenic parents that do not inherit the transgene), specifically demonstrate a lack of novel plant pest risks, even toward the chestnut blight pathogen.

As *C. parasitica* encounters the oxalate oxidase enzyme in a transgenic tree, the fungus is not killed; rather it continues to exist in saprophytic form, from which it can be easily re-isolated in artificial culture (confirmed by morphological evaluation and by re-inoculation of other chestnuts resulting in typical blight infections). In a forest setting, if such encounters occur after proliferation of the OxO tolerance trait, it is expected that the effect will be similar, *i.e.*, the blight pathogen will continue to exist in saprophytic form. Importantly, there is no selection pressure on the fungus to exert necrotrophic or parasitic effects on any living substrate in order to survive.

Further information regarding the absence of selective pressure on fungus can be found at MRID 51384802.

9. <u>Non-Transgenic Trees Will Forever Continue to be Produced</u>

Due to the inheritance of OxO by at most fifty percent of offspring from hemizygous transgenic parent trees crossed with non-transgenic trees, there will always be non-transgenic American chestnuts present, even in hypothetical restoration scenarios where transgenic chestnut trees are widely planted. While blight-tolerant trees expressing OxO allow the fungus to survive, these non-transgenic offspring likely will persist indefinitely in reduced form much as American chestnuts do today, serving as additional fully susceptible refuges (Meihls *et al.,* 2008) for blight populations, further reducing the likelihood that the chestnut blight pathogen will evolve resistance to the OxO trait.

Controlled pollinations with Darling 58 transgenic chestnut pollen have been performed according to USDA-APHIS permit conditions in permitted field plots for several years, and others have been done with pollen from legacy events not being submitted for regulatory consideration. ESF has consistently observed that no more than half the offspring from these crosses inherit the OxO transgene, confirming expected inheritance patterns from a hemizygous parent.

10. <u>No Negative Impact on Threatened and Endangered Species or Designated Habitat</u>

Introduction of OxO-expressing American chestnut into the American chestnut's natural range does not present a risk to endangered or threatened species. As part of its Environmental Impact Statement (EIS) analysis in connection with its review of Petitioner's deregulation petition, USDA-APHIS assessed the potential effects of the Darling 58 American chestnut on the environment, including an assessment of potential impact on threatened and endangered species and critical habitat.[1] USDA-APHIS reviewed the U.S. Fish and Wildlife Service's list of threatened and endangered species in the 25 states where the American chestnut historically occurred, and assessed the potential effects on terrestrial, avian, and aquatic species that could come in contact with and otherwise carry out life functions (nesting, feeding, rearing, etc.) in the vicinity of Darling 58 American chestnut trees. APHIS focused on the potential differences between the Darling 58 American chestnut and conventional American chestnuts; the potential for ecosystem effects; and the potential for gene movement to native plants, listed species, and species proposed for listing. (USDA-APHIS 2022a). As discussed further, USDA-APHIS ultimately concluded that unregulated introduction of the Darling 58 American chestnut would have no negative effect on listed species or species proposed for listing and would not affect designated habitat or habitat proposed for designation.

No differences were detected between the Darling 58 chestnut and conventional chestnut with respect to growth, reproduction, or interactions with pests and diseases (other than *C. parasitica).* Given the expected slow, natural colonization rate, USDA-APHIS found that the Darling 58 American chestnut will not rapidly invade new areas. It also found that the Darling 58 American chestnut does not present a plant pest risk, does not present an increased risk of weediness, and does not present an increased risk of gene flow. Similarly, Darling 58 American chestnut is not expected to have impacts on soil quality. Presence or expression of OxO in Darling 58 American chestnut does not pose risks to native soil. Studies conducted by the Petitioner showed there were no significant differences in colonization by ectomycorrhizal fungi in roots compared to non-transgenic controls. USDA-APHIS evaluated the potential of Darling 58 American chestnut to cross with a listed species and confirmed no sexual compatibility of any threatened or endangered species with Darling 58 American chestnut.

USDA-APHIS found no adverse effects on mammals. Indeed, the evidence suggests forest restoration with blight-tolerant OxO-expressing chestnut may mitigate the harm to ecosystem food chains that arose from the historic loss of the American chestnut hard mast during the last century. Restored, consistent hard mast yields may support increased survival of both predatory and prey mammals, benefiting the entire forest ecosystem, including threatened and endangered mammals. Similar positive effects were anticipated for invertebrate pollen eaters that feed on chestnut, and aquatic macroinvertebrates that consume chestnut leaf litter. These species in turn serve as forage

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[1] As part of its EIS review, USDA-APHIS does not consider the direct effects of pesticide use, which it views as outside of its jurisdiction and instead defers those questions to EPA to evaluate during the pesticide registration process. That distinction may be significant in the case of a conventional chemical pesticide. However, in the case of the PIP, given its passive mode of action within the tissue of a plant, USDA-APHIS' review necessarily will, in most cases, address the pesticidal effects of the PIP.

for fish and migratory birds. Slower decaying chestnut logs may increase stream channel complexity, which over time may provide additional conservation value for critical habitat for fish and invertebrates. That said, USDA-APHIS also was clear in its assessment that these were long-term benefits and, given the necessarily slow rate of restoration, the benefits would not be expected to be measurable for decades or longer.

VIII. OXO IN THE AMERICAN CHESTNUT IS NOT LIKELY TO CAUSE UNREASONABLE ADVERSE EFFECTS TO THE ENVIRONMENT, EVEN IN THE ABSENCE OF FIFRA REGULATORY OVERSIGHT

The second prong in EPA's exemption decisional criteria involves weighing the costs of imposing the full degree of oversight associated with FIFRA registration and related processes against the potential benefits. In balancing risks and benefits, EPA considers the economic, social, and environmental costs and benefits of the use of the pesticide. 85 Fed. Reg. 64308, -24 (Oct. 9, 2020).

A. The Requested Exemption for OxO Does Not Require the Public to Forego the Benefits of the Full Pesticide Registration Process

Where EPA is evaluating a proposed FIFRA exemption for a whole category of pesticide chemicals (e.g., PIPs based on sexually compatible plants created through biotechnology), the Agency must consider, among other things, the public benefit of product-by-product risk assessments, based on sufficient data, for each material in the category that the Agency has otherwise determined will present a low probability of risk to human health and the environment. EPA must determine whether full regulatory oversight, including registration procedures, is reasonably necessary to carry out the purposes of FIFRA – to assure no unreasonable adverse effects on the environment from pesticide use.

The exemption evaluation in the case of OxO in the American chestnut will be much simpler than other cases because the assessment will be completed, if at all, only after (or simultaneous with) EPA's full, product-specific, registration and tolerance exemption review and assessment processes. Those processes will include a full risk assessment with sufficient data and will consider Petitioners' intended use scenario – unrestricted, forest restoration use. That is, in granting the exemption sought by this Petition, EPA is not being asked to forego the robust FIFRA oversight provided by the registration process. That oversight already will have occurred. The Agency need only consider whether the remaining FIFRA oversight mechanisms associated with registered products are necessary to protect human health and the environment for materials that EPA has otherwise determined will present a low probability of risk. Petitioners submit that such oversight is not necessary to carry out the purposes of the Act.

B. Without an Exemption, the Registrant, Agency, and Others Would Incur Significant and Costly FIFRA Oversight and Compliance Obligations that Would Slow Restoration

Registered products create ongoing duties for the Agency, registrants, and those that work with them. These procedures – designed to regulate the distribution and commercial use of economic poisons – would be costly for those affected, and slow overall restoration efforts. These obligations would include the following:

- Each producer (grower) would first need to register as an "Establishment" with EPA's Office of Enforcement and Compliance Assurance for each physical location where the tree is produced and would be required to keep its contact information current with the Agency. 40 C.F.R. Part 167. Correspondingly, the Agency would be required to issue Establishment numbers for each of those locations and maintain records and current contact information for Establishment representatives and their agents. Each Establishment would be added to EPA's inspection and oversight workload.

- The operators of each registered Establishment would be required to maintain records tracking all transfers of whole trees and presumably transfers of propagules (budwood, pollen, and seed) (*e.g.*, shipping and receiving documents, freight bills, receiving tickets, etc.). 40 C.F.R. Part 169. This recordkeeping duty might be extended to the registrants' first tier distributors.

- The operators of each Establishment would be required to prepare and submit annual reports of the prior year's production, inventory and distribution of trees and propagules from the individual site. 40 C.F.R. Part 169. The Agency in turn would create and collect annual reports, review reports for completeness, move the information into the tracking database, and confirm all reports for all Establishments are timely submitted (or investigate and enforce where reports are late or not submitted).

- Each tree or propagule, prior to distribution, would need to be labeled with a label approved by EPA and addressing all the requirements of 40 C.F.R. Part 156. The label would be expected to include the name of the product, a statement that it had been transformed to tolerate the chestnut blight fungus, identify the OxO gene as the active ingredient and its weight percent concentration in tree tissue, the registration number, the Establishment number of the last production location, and directions for use of the tree or propagule. *See, e.g.*, Notice of Registration, C5 Honey Sweet Plum, Reg. No. 11312-8 (Sep. 30, 2104). The registrant would be required to keep the approved label current through amendments and notifications as necessary in response to changing EPA labeling policies, or other self-initiated changes to the label. EPA in turn would be obligated to review (as appropriate to the change) all modifications to the label and maintain update its public database of current and past label images and transfer information.

- Each distributor in a chain of distribution would need to maintain the approved label on or accompanying the tree or propagule until reaching the end user.

- Each year the registrant would need to pay a federal registration maintenance fee.

- Any person other than the original registrant that wanted to independently produce and distribute a tree identical or substantially similar to the Darling 58 tree for its own account would first be required to apply for and obtain its own FIFRA registration for a minimum fee of $35,182, and a nine-month review period after assembling or purchasing rights to the required data and preparing an application (PRIA B880). The Agency would need to process the application, review any additional data, and issue a new registration for the substance already determined to present a low probability of risk to human health and the environment, taking EPA resources away from other pending applications where risks are higher or unknown.

- Alternatively, persons other than the registrant could produce and distribute the tree without obtaining their own registration, but only after first negotiating and entering into a contract with the Registrant authorizing it to produce the tree as a toll producer or similar contractual production agent. The Registrant would need to oversee such agents, including their control of the registered products and any claims made for the product.

- At year 10 or so of the registration and every 15 years thereafter, EPA would be obligated to initiate and complete a new multi-year registration review proceeding for OxO in the transgenic American chestnut, just as it does for all pesticides. 40 C.F.R. Part 155. This is a very significant, resource-intensive effort for the Agency and registrants. Given their scope and the sheer number that EPA must complete, the Agency unfortunately has been unable to maintain pace with the statutory schedule for these reviews.

- The registrant will have an ongoing duty to report promptly to EPA any information it obtains regarding any adverse effects on human health, or the environment alleged to have been caused by the PIP and must submit such information to EPA. 40 C.F.R. §174.71; 7 U.S.C. § 136d(a)(2).

These ongoing FIFRA requirements place significant, ongoing costs on both pesticide registrants and the Agency.

In the case of Darling 58 and similar OxO-expressing American chestnuts, these significant requirements also would slow and make much more costly the diverse efforts by Petitioners, their individual members, and other citizen scientists to outcross and reintroduce the tree throughout its natural range. These cost, delay, and other post-registration regulation burdens on the Registrant, producers, distributors and the Agency would provide negligible additional health or environmental benefits. In these circumstances, the requested exemption from FIFRA regulation is warranted.

C. Full FIFRA Oversight of OxO in Transgenic American Chestnut Would Provide Minimal Societal Benefits that Do Not Warrant the Costs to EPA and Registrants

The post-registration FIFRA obligations outlined above can be justified in the case of most pesticides. These are traditionally economic poisons or toxicants distributed commercially in containers. They are determined not to present unreasonable risks assuming that they will be processed and used in accordance with firm operating restrictions specified on the pesticide label approved by EPA – strict control of the precise formulation, specific directions for use, including use sites, chemical application rates and application methods, warnings, precautionary statements, advice on personal protective equipment, and first aid and disposal directions. The safety of such products depends on both producers and users abiding by the terms of the label. It is a violation of federal law to use a pesticide in a manner inconsistent with its label. In those cases, the post-registration FIFRA obligations assure that products are appropriately produced, labeled and used consistent with the use scenarios EPA evaluated in its registration risk assessment.

But these provisions are totally unnecessary to assure safety of a pesticide such as OxO in the American chestnut. First, it is unlike a conventional pesticide applied to repel or mitigate a pest. It is a tree intended for widespread ecosystem restoration, including eventually natural spread into its native range. All of the pesticidal activity occurs within the tree without human intervention or control. All people can do is propagate the tree. It differs from other American chestnuts only in that it contains an additional wheat gene that is a common component of many plants commonly used as food by humans, vertebrate animals and insects. Second, EPA's registration risk evaluation necessarily will have evaluated human health and environmental risk assuming uncontrolled use -- no planting or use restrictions, no warnings, no instructions for use, no particular human conduct to avoid risk. Indeed, given the elegant design and mode of action, EPA does not need to be concerned with either displacing wild-type American chestnut populations (at least 50% of all offspring will be non-transgenic) or developing pest resistance (OxO does not create evolutionary pressure on the pest). Third, in these circumstances, the transgenic tree does not need a label and operating limitations or other EPA oversight to be safe. Indeed, if distributed as a registered product, the label would include no safety restrictions at all: no directions for use, no warnings, and no use site restrictions. The label would include only the administrative elements required by regulation for all pesticide labels - product name and number, the producer, the producing Establishment, and the infinitesimal percentage of the tree's mass represented by the OxO gene.

Once a registration risk assessment and tolerance exemption have been developed, imposing the full degree of further ongoing FIFRA regulatory oversight would cause EPA, the Registrant, and others involved in restoring the tree to incur significant costs, but those obligations would provide only negligible environmental or societal benefits. Elimination of burdensome FIFRA oversight that otherwise provides only negligible public benefit will reduce the burden on producers, who in this case are small, non-profit, research and environmental restoration entities. The exemption will facilitate a more flexible approach to distributions to develop seed increase orchards and to support the necessary regional outcrossing program. The more flexible approach also may encourage more research and development in this area of biotechnology and better enable firms of all sizes to engage in the development of these types of PIPs for environmental and societal benefit.

In addition to the benefits of an exemption to producers and distributors, the requested exemption also would reduce the burden on the Agency. As the foregoing list of continuing FIFRA oversight obligations shows, for every action required of Registrants, there is a corresponding action required of EPA. Exempting OxO in the American chestnut from full FIFRA oversight also relieves the Agency from investing further EPA resources into oversight of a product that it has already determined presents a low probability of risk in the absence of oversight. This frees EPA to concentrate its regulatory and oversight resources – registration, registration amendments, production oversight and monitoring and registration review – on other PIPs that pose higher potential risks and warrant oversight. This would be a more efficient use of EPA's resources. *Cf.* 85 Fed. Reg. at 64,325 col. 2.

D. Even if Exempt, Producers Would Continually Monitor and Report Any Adverse Effects Associated with the Pesticide

The one possible exception of negligible benefit from ongoing oversight is the duties under 40 C.F.R. § 174.71 and 7 U.S.C. § 136d(a)(2), to promptly report to EPA any information a producer obtains regarding any adverse effects on human health or the environment that is alleged to have been caused by the OxO gene. While such information is not expected, the continuing responsibility of producers to be alert to the possibility of such effects over time and to promptly report them in the unlikely event that they should arise serves as a useful sentinel function. The Petitioners have an incentive to ensure the safety and efficacy of chestnut trees distributed for restoration regardless of regulatory oversight, and detailed plans are in place to track distributions and collect regular observations on planted trees.

The fact that the OxO gene is not registered at the time would not blunt EPA's ability to act because section 3(a) of FIFRA expressly authorizes the Agency to limit the distribution, sale, or use of any unregistered pesticide, to the extent necessary to prevent unreasonable adverse effects on the environment. 7 U.S.C. § 136a(a). *See* 66 Fed. Reg. 37,771, -73 (Jul. 19, 2001). As reflected in 40 C.F.R. § 174.71(a), EPA's practice is to grant exemptions from FIFRA subject to this sentinel reporting provision, and Petitioners expect that it would follow that same approach in granting a FIFRA exemption to transgenic American chestnuts with OxO. The regulatory backstop of required adverse effects reporting should give EPA confidence that it will be aware and able to act in the face of any future, unpredicted adverse circumstances.

E. OxO in the American Chestnut Will Provide Significant Environmental, Cultural and Economic Benefits and Enjoys Wide Public Support

In addition to the immediate benefits to registrants and the Agency from the exemption, as discussed at length elsewhere in this Petition, restoration of the American chestnut to its natural range over time will provide significant environmental, cultural and economic benefits. *See* section V above, including numerous supportive comments submitted to APHIS in connection with its consideration of the Petition to Deregulate the Darling 58 and related environmental impact assessments.

IX. THE AMERICAN CHESTNUT RESTORATION PROJECT HAS BEEN ACTIVELY ENGAGED WITH THE PUBLIC AND ENJOYS WIDE PUBLIC SUPPORT

It has always been the philosophy of ESF and TACF for the American chestnut project to be as transparent as possible and listen to the public's responses (Dougherty, 2016). This has taken place via various means such as multiple field and lab tours each year, holding public demonstration plantings (under APHIS permits), conducting chestnut pollination workshops, inviting the public to help with research plantings, engaging the public at state and local fairs, and frequent presentations (with question and answer sessions) to audiences of up to 350 people at both professional and public meetings. Notable presentations include a TEDx talk sponsored by National Geographic with more than 177,000 views to date (Powell, 2013), K-12 educational programs, presenting the project in college classes, and multiple webinars with both the Fish and Wildlife Service and the National Academies of Sciences, Engineering, and Medicine's Committee on the Potential for Biotechnology to Address Forest Health.. ESF and TACF also have engaged nonregulatory governmental agencies, such as the U.S. Forest Service[2], National Resources Conservation Service, the U.S. Fish and Wildlife Service, National Park Service, and the New York State Department of Conservation. ESF and TACF also have conducted outreach activities or have formal agreements with local parks, state and private foresters, nut growers' associations, private conservation groups, and other NGOs such as the Ozark Chinquapin Foundation. ESF has reached out on multiple occasions to Haudenosaunee (Onondaga Nation) leaders in New York State to both present our project and receive feedback. TACF has a signed Memorandum of Understanding with the Eastern Band of the Cherokee Indians (EBCI) and are in planning stages for chestnut plantings on Qualla Boundary land. The USDA's Environmental Impact assessment process also included formal consultation with tribal governments, specifically to examine "…potential conflict with cultural resources on tribal properties." (APHIS 2022 p. 4-42). This consultation resulted in a total of four replies from tribes to APHIS, all of which stated they had no comments for the agency regarding the potential deregulation of Darling 58.

ESF and TACF also share and engage via written, web, and broadcast media whenever possible. Articles by research team members outside of academic journals have been featured in *The Conversation*, *The Hill*, *Scientific American*, and *The Washington Post WorldPost*. These articles and other events have in turn resulted in many local, national, and international articles, including PBS TV and radio, CBC TV and radio, *The Atlantic*, *The Wall Street Journal*, *The New York Times*, *The Washington Post*, *National Geographic*, *Scientific American*, *The Economist*, *Smithsonian*, *Ensia* magazine, the *Pacific Standard*, and the *Los Angeles Times*. Links to many of these resources are provided in the Appendix ("Additional Resources"). ESF researchers also are regular guests on TACF's "Chestnut Chat" webinar series, which typically attracts 100-300 live

[2] *See, e.g.*, MRID 51874207 (Appendix 1); and Sarah Farmer, USDA Forest Service, *Working Together Towards Chestnut Restoration* (Nov. 24, 2020).

viewers per episode, and invites questions from the audience. This type of engagement is consistent with the recommendations of the NAS Committee on the Potential for Biotechnology to Address Forest Health (NAS 2019).

This public engagement has gone beyond simply being transparent and reporting progress. Public feedback has directly informed many aspects of the chestnut research project, including its initial establishment, which genes to use (or avoid), the decision to focus only on blight tolerance rather than trying to make a "super tree," which environmental compatibility tests are most relevant to the public interest, and the decision to avoid limitations on distribution through control of intellectual property rights. The project has always welcomed and incorporated public feedback while acting on scientifically sound advice. Indeed, the decision to pursue regulatory approval and public distribution has been driven and sustained by public feedback. This public engagement will necessarily continue into the future, because restoration of the American chestnut (or other uses of bioengineering for broader environmental goals) is not a decision to be made only by researchers or regulators, but also with the input of local communities such as interested public groups and individuals who will be planting and enjoying these trees (Kofler *et al.,* 2018).

X. CONCLUSION

The foregoing Petition demonstrates that the OxO gene in the American chestnut poses a low probability of risk to the environment and is not likely to cause unreasonable adverse effects to the environment even in the absence of FIFRA oversight. As discussed in the Petition, there would be little or no benefit from ongoing traditional FIFRA regulation of this PIP after registration risk analysis. Such regulation would slow and unnecessarily hamper the restoration of the American chestnut to its natural range. In these circumstances, we urge the Administrator to find that OxO in the American chestnut and sexually compatible species is of a character which is unnecessary to regulate under FIFRA in order to carry out the purposes of that statute, and to initiate rulemaking to exempt it from such regulation.

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APPENDIX

REFERENCES

A. Primary Regulatory Documents

SUNY-ESF, Petition for Determination of Nonregulated Status for Blight-tolerant Darling 58 American Chestnut (Castanea dentata): USDA-APHIS Petition Number 19-309-01p, Docket No. APHIS-2020-0030-0002 (Jan. 21, 2020).

USDA-APHIS, Draft Plant Pest Risk Assessment: State University of New York College of Environmental Sciences and Forestry Petition (19-309-01p) for Determination of Nonregulated Status for Blight-Tolerant Darling 58 American Chestnut, Docket No. APHIS-2020-0030-8290 (June 2022). (Document ID APHIS-2020-0030-8290)

USDA-APHIS, Draft Environmental Impact Statement: The State University of New York College of Environmental Science and Forestry Petition (19-309-01p) for Determination of Nonregulated Status for Blight-Tolerant Darling 58 American Chestnut (Castanea dentata), Docket No. APHIS-2020-0030-8289 (July 2022).

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MRID 51874201 ..... Product analysis (885.1100)

MRID 51384802 .... Product analysis (885.1200, 885.1250, 885.1300)

MRID 51384803 ..... Product analysis (885.1400, 885.1500, 885.2100, 830.6302, 830.6303, 830.6304, 830.6313, 830.6317, 830.6319, 830.6320, 830.7000, 830.7100, 830.7300)

MRID 51874202 ..... Toxicology (870.1100, 870.1200, 870.1300, 870.2400, 870.2500, 885.3050, 885.3150, 885.3400, non-guideline studies)

MRID 51874203 ..... Toxicology (870.1100, 870.1200, 870.1300, 870.2400, 870.2500, 885.3050, 885.3150, 885.3400, non-guideline studies)

MRID 51384804 ..... Toxicology (870.1100, 870.1200, 870.1300, 870.2400, 870.2500, 885.3050, 885.3150, 885.3400, non-guideline studies)

MRID 51874204 ..... Toxicology (870.1100, 870.1200, 870.1300, 870.2400, 870.2500, 885.3050, 885.3150, 885.3400, non-guideline studies)

MRID 51874205 ..... Toxicology (870.1100, 870.1200, 870.1300, 870.2400, 870.2500, 885.3050, 885.3150, 885.3400, non-guideline studies)

MRID 51384805 ..... Non-target organism & environmental expression (885.4050, 885.4100, 885.4150, 885.4200, 885.4240, 885.4280, 885.4300, 885.4340, 885.4380)

MRID 51384806 ..... Endangered species

MRID 51384807 ..... Gene flow

MRID 51384808 .... individual non-target studies
MRID 51384809 .... individual non-target studies
MRID 51384810 .... individual non-target studies
MRID 51384811 .... individual non-target studies
MRID 51384812 .... individual non-target studies
MRID 51384813 .... individual non-target studies
MRID 51384814 .... individual non-target studies
MRID 51384815 .... individual non-target studies
MRID 51384816 ..... individual non-target studies
MRID 51384817 ..... Public Interest
MRID 51384818 ..... Limits of detection (analysis of samples)
MRID 51874201 ..... Product Char, Product ID test guidelines (885.1100)
MRID 51874202 ..... Product Char, Concentration of OxO, Analysis of samples (885.1400)
MRID 51874203 ..... Prod Chem/Certification of Limits (885.1500)
MRID 51874204 ..... Toxicology / Toxicity Bioinformatics & rationale (OPPTS 870.1100, 870.1200, 870.1300, 870.2400, 870.2500, 885.3050, 885.3150, 885.3400)
MRID 51874205 ..... Toxicology/Allergenicity
MRID 51874206 ..... Environmental Risk Assessment
MRID 51874207 ..... Responses to non-rejectable deficiencies
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