This response refers to the **Petition for Determination of Nonregulated status for blight- tolerant Darling 58 American Chestnut (*Castanea dentata*)** document number APHIS-2020-0030-0002, available at: <https://www.regulations.gov/document?D=APHIS-2020-0030-0002> and referred to in this comment as “the petition” or “the D58 petition”.

Submission October 19, 2020, by Allison Wilson, PhD   
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I am a molecular geneticist who has spent nearly 20 years collecting, analyzing and publishing on the unintended effects of genetic engineering (GE). I have a PhD in genetics and 6+ years of additional laboratory research experience. My research on unintended effects in GE plants includes the collection and analysis of unintended molecular effects (i.e. genomic, transcriptomic, proteomic and metabolomic), as well as unintended phenotypic effects (with a focus on harmful unintended traits having agronomic, ecological or biosafety impacts). For my various extensive scientific reviews and analyses, I rely on examples documented in the scientific literature and in petitions such as this.

My comment addresses the detrimental unintended impacts of genetic engineering on the Darling 58 chestnut, as evinced by the data provided in the D58 petition. It also discusses the unintended impacts that have not been examined and need to be addressed before deregulation of Darling 58 or any GE chestnut.

My comment first addresses the defects in the construct used to create the GE D58 event and lines. These include the use of the CaMV 35S promoter, which introduces numerous unnecessary risks; the use of the nos terminator, which is leaky and can result in aberrant chimeric transcripts; and the presence of an antibiotic resistance selectable marker transgene, which also introduces numerous unnecessary risks. Furthermore, the genomic sequence data and analysis presented in the petition, while incomplete and inadequate, indicate the presence of an unintended 600bp deletion of the native chestnut genome. Other defects cannot be ruled out at this time. Based on my extensive analyses of the presence, nature and origins of unintended effects in GE plants, I conclude that the D58 chestnut is highly likely to exhibit unintended and detrimental effects and traits. Due to its unnecessarily poor design (use of CaMV promoter, the nos terminator, and the presence of a selectable marker gene), in addition to the presence of an unintended 600bp deletion and the absence of the necessary whole genome sequences and comparisons, the petition to deregulate D58 should be rejected.

Second I address the phenotypic data presented in the D58 petition. Regardless of the event used, deregulation of any GE forest tree, including a GE chestnut, necessitates comprehensive short term and long term analysis of its unintended effects. For D58, this would include extensive –OMIC analyses, and short term and long term experiments to ascertain performance in a variety of carefully contained forest and agroforestry ecosystems, under varied biotic and abiotic conditions. Important characteristics to be tested include growth, seed production, pest and pathogen resistance, seed germination, ecological interactions etc. As noted in my full comment, and further documented in the attached references, the data presented in the D58 petition to assess the efficacy and biosafety of the D58 chestnut trees are completely inadequate – either missing, in progress, or of poor quality due to limited testing materials.

As I conclude in my full comment: Given the numerous technical weaknesses of the D58 event/line itself and the numerous scientific weaknesses of the D58 petition and its enormous data gaps, the **Petition for Determination of Nonregulated Status for Blight-Tolerant Darling 58 American Chestnut** should be denied.

Attached you will find PDF files of my full comment on the D58 Petition and the scientific references referred to in my comment.

Attachment 1. Full comment WilsonD58ChestnutComment  
Attachment 2 Al-Kaff 2000.pdf  
Attachment 3 Arpaia 2017  
Attachment 4 Cellini 2004  
Attachment 5 genewatch-uk-aphis-ge-chestnut-fin.pdf  
Attachment 6 Latham Wilson 2013a  
Attachment 7 Latham Steinbrecher 2004  
Attachment 8 Latham Wilson 2013b  
Attachment 9 PodevinDuJardin2012  
Attachment 10 SmolkerPetermann2019  
Attachment 11 Wilson 2020; not final proof14-Ch13.pdf  
Attachment 12 ENx-Genome-Scrambling-Report.pdf  
Attachment 13 Wilson et al. 2006 BSR-2-BGERvol23 .pdf