

GM Trees. Can We? Should We?

A report by GeneWatch UK

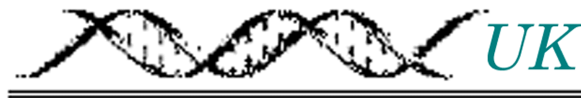


GM Trees. Can We? Should We?

A report by GeneWatch UK

February 2023

GeneWatch



GeneWatch UK

53, Milton Road, Cambridge, CB4 1XA, UK

Phone: +44 (0)330 0010507

Email: mail@genewatch.org Website: www.genewatch.org

Registered in England and Wales Company Number 03556885

Cover picture adapted from: American Chestnut Nuts with Burrs and Leaves. Photo by: Timothy Van Vliet 2004 from my Orchard in New Jersey:

https://en.wikipedia.org/wiki/American_chestnut#/media/File:American_Chestnut.JPG

This file is licensed under the Creative Commons Attribution-Share Alike 3.0 Unported licence:

<https://creativecommons.org/licenses/by-sa/3.0/deed.en>

Table of Contents

Introduction.....	1
GM trees to date	2
BOX 1. New ‘Poster Child’ GM Tree Projects.....	3
‘Virus resistant’ papaya.....	5
Non-browning apple	5
‘Herbicide tolerant’ eucalyptus	6
Risks.....	6
Molecular impacts.....	6
Gene Flow and Contamination.....	8
Ecosystem-wide exposure.....	10
Forests as complex ecosystems	11
Evolutionary responses to trees.....	13
Challenges to efficacy.....	13
Regulatory considerations	16
Consequences of potential deregulation of genome editing.....	17
Conclusion	18
Appendix 1. Summary of Global Field Trials	20
Table 1.....	20
Table 2.....	22
References	24

Introduction

The development of genetically modified (GM) trees has long been promoted, since the inception of GM industry, attempting to maximise profitability of tree industries, alter wood quality, or increase forest resilience against pest attacks. The first GM tree was a Poplar species “commercially” approved in China in 2001 (Wang et al., 2018). Wider commercialisation has thus far failed to take off globally, with commercialisation additionally limited to a few fruit tree species, none of which have been widely adopted across the agricultural sector.

The push for GM tree releases is, however, seeing a resurgence. Amidst the backdrop of a growing sense of urgency to address ecological, biodiversity and climate crises, biotech start-ups and companies are again promising GM trees as a necessary ‘scientific innovation’ to combat such problems. New projects and trials are being conducted, including the much-advertised Living Carbon project, promoted to sequester carbon for climate change mitigation, and the GM Chestnut project promoted to re-establish dwindling numbers of the iconic American tree, suffering from an epidemic of blight disease. However, behind the ‘poster-child’ projects remain those seeking to maximise industrial plantation profits, in, for example, timber, paper or pulp production, with one example being the potential imminent commercialisation of an herbicide-tolerant eucalyptus for industrial plantations in Brazil. Furthering the commercialisation and expansion of industrial plantations that are widely recognised to exacerbate climate, ecological and social problems, will further limit biodiversity, increase chemical use, and potentially deplete water supplies.

The latest projects bring the issue of GM trees once again into the spotlight. The engineering of trees, especially within wild forests, poses unknown risks to these complex, life-sustaining ecosystems. Forests are fundamental to societies across the world for whom forests provide important livelihoods, building, dietary, medicinal and cultural resources, as well as shelter. The very nature of trees, their lives spanning centuries and spreading over vast distances, their relationships both above and beneath the ground that maintain ecosystems, water and climate systems, raise significant concerns regarding the potential new roll out of GM tree varieties. This complexity is also a challenge to the potential efficacy of GM strategies performing in real world conditions. Technical limitations also remain, with the vast majority of traits and species being explored largely unchanged from the early days of GM research.

This report summarises the state of research and commercialisation of GM trees, and the risks and efficacy concerns they raise about the health of forests and the wider ecosystems they support.

GM trees to date

GM research has been conducted for decades, amidst a backdrop of widespread concern which prompted early calls for precautionary approaches to GM tree development and even international moratoria on their releases (TWN, 2006). The first field release occurred in Belgium in 1988 (Frankenhuyzen & Beardmore, 2004). Of the 205 permit applications listed at the end of 2003, 73.5% originated in the USA, 23% in other OECD member nations (in particular, Belgium, Canada, France, Finland, New Zealand, Norway, Portugal, Spain and Sweden) and 3.5% elsewhere (Brazil, China, Chile, South Africa and Uruguay) (Frankenhuyzen & Beardmore, 2004). At that time, four traits accounted for 80% of the permit applications: herbicide tolerance (32%), marker genes (27%), insect resistance (12%), and lignin modification for altered wood properties (9%). Of the tree species involved, *Populus* (poplar), *Pinus* (pine), *Liquidambar* (Sweet Gum Tree) and *Eucalyptus* accounted for 85% of applications. Insect resistance and herbicide tolerance traits that have been commercialised in agricultural settings have failed to take off in trees, despite early interest from agritech giants such as Monsanto (now owned by Bayer). Sterility traits have also been attempted, designed to prevent contamination of conventional varieties, though none have been commercialised.

Since 2003, the picture does not appear to have changed dramatically, despite poster child projects purporting to develop GM trees for societal good, such as restoration or climate change mitigation projects (Box 1). As listed in tables 1 and 2 of Appendix 1, national and regional databases of GM trial releases show a continuation of research focusing on a limited number of tree species and traits. For example, European and Brazilian permits for field trials are still dominated by altered wood property traits in poplar trees (Canadian Biotechnology Action Network and the Campaign to STOP GE Trees, 2022). According to the National Academy of Science, the United States, a centre for GM tree development, the US had approved 387 permits for trial release, 220 for non-fruit species and 167 for fruit trees (up until 2018), with a higher focus on fruit trees than other global regions. Fruit tree traits overwhelmingly focus on pathogen and pest resistance, altered product quality and altered flowering, while non-fruit tree traits still focus largely on altered wood properties for industrial purposes and herbicide-tolerance (see Tables 1 and 2). Up to 2021, 51 permits have been issued in the US for poplar species (1989-2021), 19 for altered wood product quality, 16 for altered agronomic quality traits (e.g., altered flowering, increased photosynthesis), and 11 for fungal resistance (see Table 1b). Despite two decades of GM poplar research, developers are still working on the same traits that were initially investigated, including sterility and altered lignin, though without any commercialisation success for sterility or lignin to date. Twenty-one eucalyptus permits have been issued (from 2007-2019), all from the company ArborGen (altered lignin, altered flowering and cold tolerance traits). Fourteen walnut permits have been issued, all but one to the University of California, with 10 of the 11 developed for bacterial resistance. 34 permits have been issued for apple trees, 17 of which are for altered product traits such as non-browning, and 11 for altered agronomic quality such as increased cold tolerance or altered flowering. Nine plum tree permits were issued (from 1991-2014), either for altered flowering and/or pathogen resistance.

China had conducted 78 field trials up to 2013, (including 34 poplar trials). More recent data is not available. India has recently embarked on its first GM tree trial, in 2021, planting rubber trees designed to tolerate colder conditions, in order to cultivate the crop in regions not normally suited to rubber plants (The Hindu, 2021). Since 2003, Europe has issued 27 permits for forest trees (Table 1a), all but two in poplar trees with a focus on altered wood quality traits, with two birch trials conducted in Finland. Sweden is a European centre for tree trials, with 13 of the 27 being conducted there. Malaysia has also performed several trials to develop GM rubber plants that produce medicinal products (Table 2).

BOX 1. New 'Poster Child' GM Tree Projects

The GM Chestnut (*Castanea dentata*) project set to take place in the US, is being promoted to restore the iconic GM Chestnut populations described as 'functionally extinct' as a result of the invasive spread of fungal blight (and root rot). In 2020, State University of New York College of Environmental Science and Forestry (SUNY-ESF), applied for 'non-regulated' status to the US authorities, although a final decision (as of February 2023) is yet to be made. If approved, it would be the first modified trait destined for wild forest ecosystems. Moreover, it would be the first to be sold under the guise of ecological restoration. If granted non-regulated status, the researchers state that the trees will be available for "*non-profit distribution to the public, and to groups including private, indigenous people, the state, and federal restoration programs, depending on the goals and preferences of these various groups.*" (State University of New York College of Environmental Science and Forestry, 2020).

The tree was modified to withstand infection with the fungal pathogen, *Cryphonectria parasitica*. This was done by introducing a wheat gene (encoding for the enzyme oxalic acid oxidase) that metabolises a toxic product of fungal infection (oxalic acid) that damages the trees. The trait is designed to reduce levels of oxalic acid, allowing the tree to tolerate, but not resist or kill off, infection.

The engineering of a wild chestnut has raised a variety of concerns regarding how it may impact surrounding forest ecosystems. Understanding the impacts would take decades of assessment, yet biosafety and efficacy data on the trees are extremely limited (GeneWatch UK, 2021), making any potential approval at this time seriously premature. The wheat gene has never before been used outside the lab for engineering purposes, and the inclusion of an antibiotic resistance gene will be the first outside of agricultural settings, raising concerns about their impacts if and when genetic contamination inevitably occurs. Indeed, there are still an estimated 2 million mature chestnut trees still growing in the United States (Davis, 2021).

In comments submitted to US regulators on the potential release, the Canadian Chestnut Council, a scientific and charitable organisation, disagreed with the concept that the GM tree is "*a restoration tree*", and stated that "*its release will contaminate the remaining population of American Chestnut and result in possible greater harms*" (Canadian Chestnut Council, 2022). Their efforts to restore the populations include breeding programs with the planting of 32,000 trees, the planting of grafted trees or seedlings near to geographically isolated trees; as well as programs to identify, preserve and enhance populations, such as testing for genetic contamination, health status, and ensuring sufficient light exposure to increase survival. The Council raised concerns over the lack of regard for their aggressive and painstaking restoration efforts by those proposing the GM release.

Davis (2021), a scholar of the Chestnut, also challenges the key notion of the species being 'functionally extinct'. While this framing may serve to galvanise support for experimental releases, it raises important biosafety considerations. Introducing a GM Chestnut may serve as a novel risk to restoration efforts of long-established native breeding programs. It would be the "*ultimate unintended consequence, and a tragic irony, if existing surviving trees, would end up being contaminated with transgenic material*". Furthermore, GM trees potentially serve as a fungal reservoir for the pathogen. As stated in the permit application regarding the 'blight tolerant Darling 58 variety "*In the case of a tolerant host without any toxicity mechanism, all hosts essentially function as refuges...*". It is indeed possible that large-scale plantings, could serve merely to increase blight spread. Moreover, efficacy questions loom over the project. Tolerance to blight may decline as the tree ages (Davis, 2021). With current trial data gathered from trees up to the age of 3 years old, how blight tolerance withholds over the course of the trees' life-span is not yet established.

The American Chestnut is increasingly plagued with numerous invasive and native pests. Trials on conventional trees infected with blight show mortality rates of 12-70 % resulting from other diseases, insect or animal damage such as ink disease (Clark et al., 2014). Consistently, in GM trials, nearly all seedlings (conventional and GM) succumbed to ink disease. There remain questions regarding potentially poor resilience of the trees in the context of multiple stressors such as pathogens, pest attacks or climactic changes that may compromise resistance mechanisms in the trees (GeneWatch UK, 2020; Woodcock et al., 2018). Further, the development of GM trees from a single clone reduces genetic variation, which can increase susceptibility to stressors. It is also yet-to-be understood how modifying oxalic acid levels may impact the tree, which possesses functional roles within plants, involved in various processes from calcium regulation, as well defence against insect pests, pathogens and grazing animals (Prasad & Shivay, 2017).

The project has received sharp criticism for its 'feel good factor', and a potential to be a Trojan Horse that leads the public and regulators into accepting wider-scale roll outs of GM trees, particularly those that are seeking to maximise profitability of industrialised monoculture systems.

BOX 1 (contd.) New ‘Poster Child’ GM Tree Projects

The Living Carbon Project is being promoted by its venture capital developers as aiming to make the “*Tesla of trees*”. These trees are being promoted as a carbon off-setting technology, capturing carbon by engineering poplars to have higher photosynthetic rates and thus grow faster, in an attempt to “*help combat the looming consequences of climate change*” (Living Carbon Team et al., 2022), and also for industrial wood production. Carbon credits are already being promoted despite the lack of any available product, amidst a backdrop of wider criticisms of carbon offsetting mitigations, many of which have been described as seemingly “*worthless*” (Greenfield, 2023). The project was recently bought by Frontier, with a ‘market commitment’ launched by Stripe, Alphabet, Shopify, Meta, and McKinsey Sustainability (Frontier, 2022).

The first field trials were only planted in July 2021, in collaboration with Oregon State University and are yet to be analysed. Greenhouse experiments were reported in un-peer-reviewed data to increase biomass by up to 53 % in young trees, though this effect was temperature dependent, with no difference seen at 40°C (Living Carbon Team et al., 2022). How traits that are environmentally mediated may perform in real world settings remains to be seen. Living Carbon themselves conclude in their un-peer-reviewed report that it is “*a challenging goal to engineer trees to make a meaningful impact on climate change*”.

Fundamental questions regarding the underlying rationale of the project have been raised. First and foremost is the issue of land availability required to plant sufficient trees to allow for any carbon offset. Second, how well can levels of ‘carbon off-setting’ be predicted for a project that will take at least 30 years for the trees to mature? Nonetheless, pre-purchases are already available by Frontier. Third, engineering trees to grow faster risks making the trees weaker structurally, with potential loss in resilience. While the aim is to improve carbon capture, any unintended effects that may undermine the survival of the tree and lead to die-offs would result in carbon being released back into the environment. Trees will need to be harvested at a currently unknown, correct time to prevent carbon going back into the atmosphere. As the Head of Trees at Kew Gardens UK, Kevin Martin, recently warned, “*we need to increase protected forests, not introduce more risks and potentially less resilience*” (BBC, 2022).

Despite the large number of trials across the world to date, GM trees have not been widely commercialised across the globe. The lack of adoption is a signal of potential efficacy and risk problems that have hindered development thus far. As detailed below, the most advanced GM projects have suffered various pitfalls from low adoption rates to efficacy problems. Our incomplete knowledge around genetic determinants of tree traits, as well as technical difficulties in engineering trees, are also limiting potential applications for novel traits beyond those that have been extensively studied for decades without success e.g., lignin modification.

“*Insecticidal*” Poplars

The first “commercial” approval of a GM tree was granted in China as early as 2001 (Wang et al., 2018). Poplar trees were first tree species to be modified (Fillatti et al., 1987) and soon became the model system in forest biotechnology (Hjältén & Axelsson, 2015). In China, engineering poplars has been ongoing since the 1990s, with a reported 22 GM varieties developed and approved for testing (up to 2018). Only two however, have been “commercialised” amidst a backdrop of biosafety and consumer concerns (G. Wang et al., 2018). The two transgenic poplar varieties, both trialled from 1996-2001, include a poplar 741 variety that carries an insecticidal Bt toxin (Cry1a), and another insecticidal gene called *API*. The second product is a *Populus nigra* variety that carries the Cry1A toxin only. According to the pro-biotech resource ISAAA.org, the adoption rate for the GM poplar appears to be low, with only 543 hectares reportedly planted in 2016 (ISAAA, 2017). The long-term requirements for assessing safety have led to a cautious approval approach by the government (G. Wang et al., 2018). These researchers note that fully assessing the risks of Bt poplars prior to commercialization poses great challenges, requiring multiple studies spanning numerous sites and successive generations of trees to evaluate ecological performance.

Beyond GM Poplars in China there are yet to be any other commercialised forest trees being cultivated anywhere in the world. However, some fruit trees have been commercialised, including 1. GM papaya in China; 2. GM papaya in Hawai'i (United States); 3. Non-browning apple in the USA and Canada.

'Virus resistant' papaya

Both GM papaya varieties are modified to exert resistance to the Papaya Ring Spot virus (PRSV), a virus carried by aphids that has been spreading internationally from Hawaii since the 1960's. In China, Huanong No.1 papaya, commercialised in 2012, was modified to tolerate four strains of the aphid-transmitted PRSV, with adoption rates of 14 % reported in 2018 across four provinces. Within 6 years, problems began to emerge that are now considered to be a threat to the survival of the variety due to the evolution of resistance. The presence of resistant strains of viruses was reported in two provinces growing Huangong No.1 (Mo et al., 2020; Wu et al., 2018), with the strain infecting plantations identified as a novel lineage present on transgenic crops, now a threat to the survival of the variety. Zhao et al., (2015) showed that the presence of PRSV increases gradually with time, with trees thus acting as a potential reservoir for resistant viral populations. Papaya leaf distortion virus (PLDMV) has also impacted plantations (Mo et al., 2020). Infection with PLDMV occurred immediately in 2012. Mo et al., (2020) hypothesise that the virus has evolved to be transmitted readily by aphids like PRSV. In 2020, more than 10 % of Huanong plantations had virus like symptoms (all viruses), with 10-40 % disease incidence, resulting in significant crop losses (Wu et al., 2018).

The 'SunUp' Papaya cultivated in Hawai'i was first made available to farmers in 1998, developed by researchers based in Hawai'i and Cornell University, US. The variety reached high adoption rates, reportedly 85 % by 2016 (Gonsalves, 2016), but the adoption came at significant cost to the papaya industry, with losses in export markets of \$15 million worth of fruit to Japan. Widespread contamination of organic and non-GE varieties has also been reported (see below). The product is not grown outside Hawai'i, and appears ill-suited for wider cultivation due to insufficient protection against other strains of PRSV (Tennant, 1994), showing no symptomatic protection against isolates from Brazil and Thailand, and limited protection against isolates from The Bahamas, Florida and Mexico. PRSV populations are highly genetically diverse (Jain et al., 2004), which likely limits the effectiveness of transgenic strategies to control the pathogen. Brazil and Mexico remain in the top 5 global producers despite the lack of available GM products for their region.

Non-browning apple

The non-Browning 'Arctic' apple, developed by Canada-based Okanagan Speciality Fruits, was approved for cultivation in the US and Canada in 2015 and is now on the market. The apple varieties, 'Arctic' Golden Delicious, Granny Smith and Fuji Apples, are designed to not brown after cutting, in a purported bid to increase apple consumption. Though recent figures do not appear to be available, adoption rates appear to be low, with a reported 500 hectares being planted in 2019, a 0.4 % adoption rate, two years after the first planting season in 2017. The apple utilises RNA interference 'gene silencing' technologies to suppress, or 'silence' the expression of a gene (see risk section below). Low adoption rates combined with the existence of naturally occurring non-browning apples in the US market (FirstFruits, n.d.) undermine any claims that this trait is either necessary or popular amongst consumers or growers.

'Herbicide tolerant' eucalyptus

Brazil has not yet commercialised GM trees, though it is potentially on the brink of cultivating a recently approved (2021) glyphosate herbicide-tolerant eucalyptus variety, intended for plantation production of pulp products. Eucalyptus plantations are already a source of environmental and social problems that expand the industrialised plantation economy into indigenous and traditional territories and natural ecosystems, reducing biodiversity and expelling people from their lands (Branford, 2021). Indeed, the Cerrado savannah region is reportedly home to a third of Brazil's biodiversity, yet it has been hugely targeted by agrobusiness in recent decades, with half of it now converted into monoculture systems that are home to GM agriculture as well as eucalyptus plantations.

Suzano, the largest pulp exporter globally, is a major player in the GM tree push in Brazil, with its subsidiary FuturaGene developing the new GM eucalyptus that it is promoting as a reforestation climate solution and a supposed strategy for carbon capture. The latest approval builds on previous attempts by Suzano to introduce a different variety of GM eucalyptus that was modified to grow faster, but never reached commercialisation despite gaining approval in 2015. This GM tree failed to replicate the initial findings claiming a 20 % increase in growth rates when planted in different sites across the country (Ledford, 2019). This coupled with a lack of interest from the market in purchasing the trees led to the downfall of the project, and the entry of the most common and controversial trait of the GM industry – herbicide tolerance. The herbicide tolerant (HT) trait allows the GM crop or tree to survive blanket spraying with the associated herbicide. While eucalyptuses are fast growing, and able to grow in poor soils which are attractive traits for environmental goals, they may also lead to soil acidification, increased water use, soil erosion, soil nutrient depletion, and reduced biodiversity (Bayle, 2019; Branford, 2021).

Plantation agriculture as a whole, has a higher density but lower diversity of trees than natural forests (Lindenmayer & Hobbs, 2004), which has in turn been associated with reductions in the number of species of several animal groups, such as arthropods, reptiles, amphibians, and birds (Faruk et al., 2013; Fork et al., 2015; Glor et al., 2001; Zurita et al., 2006). Suzano's claims that plantations are destined for poor and disused land does not hold water, with plantations met with intense campaigns by Brazilian communities and environmental movements to protect against eucalyptus expansion (Branford, 2021). Most crucially, the planting of herbicide-tolerant trees will result in blanket chemical spraying, with clear implications for biodiversity as well as wider environmental pollution of land and water systems.

Risks

Molecular impacts

GM is well established to have unintended impacts at the molecular level, with both older transgenic as well as more recent genome editing techniques. The risk of unintended effects within wild tree species and forest ecosystems, however, may well exacerbate these risks beyond what has been experienced to date.

Older GM techniques either use the bacterium, *Agrobacterium tumefaciens*, as a vector for new DNA which is being introduced, or coat the DNA to be inserted on minute gold particles and fire it into cells. Many, but not all, GMOs produced using such GM techniques are 'transgenic', i.e., they contain genetic material into which DNA from an unrelated organism has been artificially introduced. Newer genetic engineering techniques have been developed which are collectively known as 'gene editing' or 'genome editing'. These also use

the bacterium, *Agrobacterium tumefaciens*, or minute gold particles, to transfer material into cells. Enzymes and templates are introduced into the cell of a plant or animal to cut and edit its DNA at a targeted location, using the cell's own repair mechanisms. Some simpler forms of gene editing are not transgenic. However, the vast majority of genome editing still requires the introduction of transgenic material, including transgenes encoding for the editing machinery. This is discussed further in the regulation section.

In GM crops, unintended impacts of the GM process, as well from the introduced trait have been widely observed (see for example reviews by ENSSER, 2021; Wilson, 2021). Such impacts include genetic changes, as well as disturbances in gene expression, protein and metabolite levels. Indeed, GM crops to date have suffered a wide variety of unintended molecular effects (see reviews, e.g. Wilson, 2021) and commercialised crops suffer a variety of unintended effects including on fitness, seed germination, weed suppression, pest resistance, (non-)drought-tolerance, height, yield and flowering time, as well as compositional differences (reviewed by ENSSER, 2021; Wilson, 2021). Such risks are recognised in the regulatory context, with both national and international regulations e.g. the Convention for Biological Diversity's Cartagena Protocol on Biosafety, requiring assessment at the molecular level (Mackenzie, n.d.). Current assessments have however, been regularly criticised for being permissive and insufficient to detect and ensure against potential risks (e.g. Hilbeck et al., 2020), and in the context of tree releases may pose particular concern given characteristics of potential gene flow across large temporo-spatial scales.

Consistent with GM crops, unintended molecular impacts have been documented in GM trees, including commercialised papaya varieties (Yue et al., 2022), as well as the experimental GM Chestnut (Davis et al., 2021). The Chinese papaya has numerous unintended changes to the genome (deletions, insertions, translocations) as a result of the engineering process (transgene delivery (transformation), and the tissue culturing). Moreover, the study concluded that altered gene expression patterns seen in both the SunUp and Sunset genomes may have arisen from the activation of mobile genetic elements, which are "*potentially a rich source of variation on which selection may operate*" (Yue et al., 2022). Mobile genetic elements are types of genetic material that can move, or hop around in the genome, causing mutations and epigenetic reprogramming as they re-insert themselves into new genomic locations. Such genetic elements are thought to have played an important role in plant adaptation and evolution (Lisch, 2013), suggesting that such unintended changes may also potentially have evolutionary consequences for GM tree releases, particularly if they are released into wild forest ecosystems. Molecular changes in the GM Chestnut have been observed (genetic inversion), the consequences of which were not assessed, but instead assumed by developers to lack biological significance (State University of New York College of Environmental Science and Forestry, 2020).

GM trees that utilise RNA interference (RNAi) mechanisms to alter traits, provide another mechanism by which unintended molecular changes may occur. RNAi is a naturally occurring cellular system that functions to control gene expression in an organism, and is thus taken advantage of by GM developers to turn off, or 'silence' genes, in order to change traits. The Living Carbon project is, for example, introducing a gene that encodes for an RNAi molecule (technically termed a double-stranded RNA), to try to reduce the expression of a protein involved in photosynthesis. RNAi techniques have also been used in commercialised papaya and non-browning apples, and their use has been attempted several times to alter lignin content in pine trees (Chanoca et al., 2019). RNAi suffers from intrinsic risks including the potential "off-target" silencing of other genes within the target organism, as well as unstable and variable silencing (Leplé et al., 2007; Van Acker et al., 2014a; Voelker et al., 2010). Unexpected impacts of silencing the target gene have also been documented in RNAi GM poplars developed to induce flower sterility. This resulted in reduced growth as well as altered leaf traits thought to result from the silencing of the target

gene (Klocko et al., 2021). Unstable silencing was also observed. Without knowledge of all the genes that exist in all the potentially exposed organisms to RNAi molecules in the trees, one cannot rule out that RNAi molecules may exert impacts including silencing of genes in non-target organisms.

GM trees increase the risk and uncertainty of how such molecular impacts may impact the target trees and any exposed organisms, as well as the functioning and sustainability of forest ecosystems. The release of GM organisms into wild ecosystems is currently forming a major part of regulatory discussions, with the advent of GM projects such as those targeting wild insect and fish populations. Unlike GM commodity crops, trees, and forest trees in particular, tend to be genetically diverse. Unintended impacts of GM at the molecular level are mediated by the genetic background of the target organism, resulting from interactions of introduced changes (both intended and unintended) with the genome of the modified organism. This is exemplified by the report of a GM rice variety that displayed dwarfism when the trait was introduced to a novel genetic background (Bauer-Panskus et al., 2020). Moreover, in the case of using GM technologies to restore tree populations, any fitness costs resulting from unintended effects may undermine recovery of a species over a large spatio-temporal scales.

Current risk assessment protocols in general are not in step with the complexity of molecular impacts that can occur when attempting to modify complex, long-living organisms destined to propagate in wild ecosystems.

Gene Flow and Contamination

A consistent concern for GMOs in general has been the potential for contamination of non-GM food supplies in the market place, as well as the genetic contamination via gene flow, of modified traits from a GM organism to a non-GM organism following reproduction.

Genetic contamination is a big problem for forest trees, with the ability to control gene flow practically impossible due to the long-range and varied dispersal of pollen or seeds by wildlife (e.g., mammals, birds, insects) and weather systems (wind and rain), as well as vegetative propagation (asexual reproduction from regeneration of plant tissue, e.g., roots, stems) in some species. Moreover, reproductive tree characteristics such as high fecundity together with seed dormancy, high seed viability and dispersal, further complicate these risks, increasing uncertainty regarding the ability to control contamination events. Gene flow within the species, as well as inter-species gene flow via horizontal gene flow or hybridisation has been one of the major concerns around potential GM tree issues. Risks are further exacerbated by the longevity of tree species.

Gene flow is difficult to measure, influenced by the reproductive biology of the plant, pollen survival rates, climatic, abiotic and management factors, the large scale of potential dispersal, impacts of the genetic change, as well as inherent difficulties in detecting low levels of introgression of transgenes over such large spatial scales. As stated by the US National Institute of Science Report (2019) on tree biotechnology, "*This problem is greatly magnified for trees, which are typically outcrossing, can disperse pollen and seeds over dozens or even hundreds of kilometers (Slavov et al., 2009; Williams, 2010), and may contribute pollen for centuries*" (Committee on the Potential for Biotechnology to Address Forest Health et al., 2019). Long-distance pollen travel has been documented in pine with germination rates of 2-57 % after dispersal distances of 3-41km for pine pollen (Williams, 2010), and high potential for long-distance gene flow between fragmented populations of pine by wind-mediated dispersal (Jiménez-Ramírez et al., 2021). Chestnut trees are known to produce huge amounts of pollen, that have been shown to travel distances of up to 40 km (Peeters & Zoller, 1988). They were also recently confirmed to be highly pollinated by

insects in Europe, providing a major pollen and nectar resource for a large variety of wild insects, including honeybees, at a critical period of the year when resources are scarce (Petit & Larue, 2022).

Genetic contamination has already been reported for all but one of the commercialised trees to date, including during field trials. Genetic contamination was reported just two years after commercial planting of GM poplars in China (New Scientist, 2004). The lack of licencing of the GM trees further compounded the ability to control the problem. Hawai'ian papaya cultivation similarly reportedly led to widespread contamination of non-GE papaya, including organic and feral populations (Bondera & Query, 2006). GM papaya trials in Thailand (of the US developed SunUp varieties) also resulted in genetic contamination (Davidson, 2008) and subsequent import bans from the EU with significant economic impacts (GMWatch, 2004). This occurred amidst warnings from concerned environmental groups, despite assurances that trial biosafety protections would be sufficient to prevent spread. Freedom of information requests made about the GM Chestnut project also raise questions regarding the biosafety standards of the trials, with some plants being reported as missing (Davis, 2021).

Pollen-mediated gene flow from transgenic trees has not been widely assessed thus far in countries like the US, where field trials are generally required to bag flowers to prevent spread (Committee on the Potential for Biotechnology to Address Forest Health et al., 2019). However, lapses in protocol have been observed for GM Chestnut trials, reducing confidence in how much trials have inadvertently led to contamination events, as has been previously documented for GM crops (Price & Cotter, 2014).

The spread of transgenic traits, as well as unintended genetic changes, comes with both known and unknown risks. Intended traits such as reduced lignin, that is associated with weakened tree structures and subsequent growth and pathogen/pest defence problems, may spread from GM plantations to wild populations, potentially risking forest resilience. Faster growing trees also risk increasing resource use requirements, e.g., water, reducing resilience and exacerbating, rather than addressing, climate problems. Unintended impacts such as those already associated with agricultural traits, such as Bt insecticidal toxins, may go on to impact non-target organisms and thus forest biodiversity.

Contamination of wild populations, including potentially fruit relatives, also adds a deeper level of complexity to risks of unintended effects. Trees, unlike annual crops, have a low level of domestication that may allow them to compete and persist in wild ecosystems, with most breeding programs to date only progressing through a few generations (El-Lakany, 2004; FAO, 2004; Isik et al., 2015). Any unintended effects of a modified trait at the molecular level, such as disturbances to the genome, may also be impacted by the genetic background and wider environmental interactions.

Contamination raises particular concern for conservation projects attempting to restore species for conservation goals, such as the GM American Chestnut project. As Davis (2021) warns, an ultimate unintended consequence of a GM restoration project, would be the jeopardising of current restoration efforts already working with native varieties, by permanently altering species germplasm forever.

Another source of contamination is the *Agrobacterium* vector itself, the bacteria used to deliver the transgenes to the trees during the development process. *Agrobacterium* is a soil bacterium that infects plants, causing tumours, but has been reported to also infect mammalian (human) cells, presenting a biosafety risk to both human and environmental health (Adnan et al., 2013; Kunik et al., 2001). The spread of *Agrobacterium* was detected in soils of a Chinese poplar plant in greenhouse conditions after 1 month, though little data exists from environmental releases (G. Wang et al., 2018).

Ecological consequences may result from transgene flow in unexpected ways. For example, developers have long insisted that contamination of wild relative species of crops modified to carry herbicide resistance genes is irrelevant to environmental risk. The claim is that any genetic contamination beyond crop fields where herbicides are not applied will not have any biological impact on the organism or exposed organisms. However recent data showed that genetic contamination of wild cotton relatives resulted in ecological consequences. Interplay between the herbicide-tolerance trait and other pathways involved in nectar production resulted in reduced nectar in wild plants, and its subsequent association with ant species that ordinarily protect the plant against herbivore damage. Increased herbivore damage raises serious concerns for the impact on evolutionary processes of wild cotton species located in their centre of origin in Mexico (Vázquez-Barrios et al., 2021).

Ecosystem-wide exposure

The longevity of trees means that they provide resources to many species over decades and sometimes hundreds of years, allowing forests to harbour substantial biodiversity (Brockerhoff et al., 2017). The diversity of forest life thus exposes large parts of the ecosystem via fruit, pollen, live and dead matter both above and below the ground across large spatio-temporal scales.

Chestnuts, for example, provide a rich source of pollen to insects including honeybees. Their nuts are also widely consumed by numerous animals from bear species to birds, while leaves provide a resource to insects such as numerous species of moths. Below the ground the species is also a host to beneficial ectomycorrhizal fungi, including species thought to mediate water relations, and increase soil carbon (Bauman et al., 2018). Early field trials of GM aspens with altered auxin hormone levels found that one line showed reduced mycorrhization, thought to be a result of unintended molecular effects that may have led to unpredictable and unexpected changes (see Frankenhuyzen & Beardmore, 2004). Fungal-tree cross-kingdom communication is now understood to allow beneficial fungal colonisation of trees, via suppression of the tree immune systems (e.g., via fungal release of RNA molecules) (Wong-Bajracharya et al., 2022). Such complexities in relationships may lead to risks from traits such as those designed to resist fungal pathogens, for example.

Risks to soil may arise through several mechanisms, including direct effects of the novel trait and indirect effects due to unintended alterations to the plant as a result of the GM process. Moreover, changes to management practices, e.g., use of chemical inputs, or expansion of artificial plantations may potentially alter the soil ecosystem. For example, GM trees such as the Brazilian eucalyptus express the transgenic material in the roots, with potential accumulation of any novel or altered root exudates and residues over time. Another concern is how the tree litter such as leaves, wood, bark, etc., will impact soil (Lebedev et al., 2022). Bt toxins expressed in commercialised crops are also widely detected in root exudates and soil, even for up to 3 years (Liu et al., 2021), and have been shown to induce toxic metal uptake by crops (Zhang, 2015), and to be linked to adverse soil impacts, including toxicity to non-target organisms such as earthworms (Zwahlen et al., 2003), and mycorrhizal fungi (Chen et al., 2016). Transgenic Bt plants have also been shown decompose less in soil than non-Bt plants, the ecological consequences of which are currently unclear (Flores et al., 2005).

Altering lignin levels, which are consumed and degraded by soil microorganisms, may also alter the soil ecosystem, and also carbon release. Trunks from trees modified to have reduced lignin levels have previously been shown to decompose more rapidly. Reducing lignin levels in poplars has already demonstrated shifts in the bacterial community, hypothesised to result from an unintended altered abundance of particular phenolic metabolites in the xylem of the trees (Beckers et al., 2017).

Beyond fruit trees, forest trees including chestnuts also provide a source of food for people, as does honey from eucalyptus, raising implications for food and feed safety. Risk assessment documents show the presence of transgenic products (antibiotic resistant gene product) in the honey from GM eucalyptus, the implications of which have not been empirically tested, but instead presumed safe. This also has socio-economic implications that threaten organic or non-GMO markets, since levels of transgene product are above the contamination threshold allowed for organic certification (National Technical Biosafety Commission, 2015).

Forests as complex ecosystems

GM trees pose some distinct risks from current GM technologies that have thus far been overwhelmingly restricted to a narrow range of commodity crops. While GM crops have consistently raised public concerns over the safety and corporatisation of the food system, GM forest trees may directly impact wild forests that are essential for the survival of our planet. Forests (particularly tropical ones) are global centres of biodiversity, with tree biodiversity (not just tree numbers) reducing climate change impacts by increasing carbon capture, regulating drought, pest attacks and fire outbreaks (Beugnon et al., 2022). Trees and forest ecosystems provide vital ecosystem and societal roles including preserving soil fertility, providing shelter, oxygen, food, protection from floods, fires, pollution, wind, landslides and soil erosion, carbon storage, supporting both terrestrial and aquatic systems, and providing an array of dietary, medical and culturally significant resources for people across the world. Healthy forests sustain ecosystems and societies over time and space.

The introduction of herbicide-tolerance, or insecticidal properties, into complex ecosystems risks increasing the already known adverse impacts associated with the use of these traits in GM crops. The use of herbicides as intended with the current Brazilian herbicide-tolerant eucalyptus, involves blanket spraying of herbicide, with expected adverse impacts on biodiversity. The unintended impacts of herbicides such as glyphosate are now well established, both in terms of human and environmental health, though new data continues to emerge (see GeneWatch UK, 2022), including toxicity to non-target organisms, loss of habitats for species such as the Monarch butterfly in the USA, and rapid rises in resistant weed species with concomitant increases in pesticide usage. A recent example is a new study reporting that glyphosate herbicides can directly induce mortality in insects, thus exerting potential pesticidal as well as herbicidal properties (Defarge et al., 2023). Glyphosate herbicides have been detected in soil, rain, air, surface water as well as groundwater samples, contaminating water systems that are regulated by forests (Battaglin et al., 2016; Mas et al., 2020; Rendon-von Osten & Dzul-Caamal, 2017).

In the case of insecticidal properties, the toxicity of Bt insecticides to soil organisms such as earthworms and mycorrhizal fungi that are vital to forest ecosystem health could have major implications that are very difficult to assess and predict. There is extensive evidence that the target pests evolve resistance to GM Bt crops (Alvi et al., 2012; Dhurua & Gujar, 2011; Faretto et al., 2017; A. Gassmann, 2021; A. J. Gassmann, 2016; A. J. Gassmann et al., 2011; Gunning et al., 2005; Gutierrez-Moreno et al., 2020; Ludwick et al., 2017; Tabashnik & Carrière, 2017; Wan et al., 2012; Zhu et al., 2015). In GM trees, Wang, et al. (2018) report that no resistance has yet been found in Bt poplars, but note that this could be because insect pests may also feed on other plants, diluting the effect of the toxins, or because the test plots used were relatively small. They state that, if large plantations of GM poplar are permitted in the future, it is likely that selection pressure will be much higher so that pests are more likely to evolve resistance. Intended reduction in target pest numbers may also have unintended impacts such as rises in secondary pests, as widely experienced already with Bt crops such as cotton for example, leading to increased pesticide use (Hagenbucher et al., 2013; Kranthi, 2014; Lu et al., 2010; Nagrare et al., 2009; Nair & Bhardwaj, 2015; S.

Wang et al., 2008; P. Yang et al., 2005). Wang et al. (2018) cite research published in Chinese which shows the number of herbivorous insects was significantly lower in transgenic poplar 107, whereas the number of sucking insects was significantly higher. This may illustrate a potential problem with secondary pests. Industrial agro-ecosystems are to some extent, easier to manage and control, due to limited biodiversity and the use of management practices to mitigate unintended effects. However, this cannot be relied upon for wild forest ecosystems across the time-scales required to prevent or control for such unintended harms.

Important questions are also raised by our increasing awareness of the extent to which tree relationships with soil microorganisms are vital for forest ecosystems. Seminal work on the fungal networks that live in symbiosis with tree species is revealing their yet-to-be fully understood role in providing live communication networks between trees, even between the mother and its offspring (Simard, 2021). These connections between roots and soil life are now thought key for plant nutritional uptake, communication, family networks, resilience to stressors such as salinity, drought, heavy metals and disease; other microbiome relationships, with interdependent and far-reaching relationships, with for example aquatic life, e.g. salmon, feeding and sustaining mycelial networks, and by extension, trees (Ryan, 2017; Simard, 2021). The complexities of forest ecosystems go beyond current human understanding, let alone our ability to predict or control any potential impacts, raising alarm regarding both the intended as well as unintended impacts of GM tree traits.

The long-lived nature of trees also means that any introduced risks may only become visible, or be maintained, across hundreds of years, outliving the developers of the trees, and affecting the species composition of forests for centuries. Some pine trees do not reach reproductive age until they are 20 years old, and can live for over 1000 years (Committee on the Potential for Biotechnology to Address Forest Health et al., 2019). Not only does this have implications for the exposed ecosystems, but also future generations of people who may have to bear the costs of unexpected or unintended effects (Committee on the Potential for Biotechnology to Address Forest Health et al., 2019). Ecosystem or food web effects are difficult to study due to high complexity (Lim & Traavik, 2007). Ecosystem impacts of invasive animal species have been documented to take decades to become visible, for example. The longer life-span of trees introduces yet more uncertainty, such as, for example, the potential invasiveness of the GM Chestnut that, in effect, is designed to replace the native populations.

The expansion of GM trees also risks the expansion of commercial forests and monoculture plantations that can pose a threat to the sustainability of healthy, wild forest ecosystems. Tree plantations are already linked to increased chemical use, which will form an inherent part of any herbicide-tolerant tree cultivation practice, with obvious implications for biodiversity impacts and thus forest ecosystem and wider climate function. Plantations, as such, cannot be thought of as a healthy alternative to natural forests. Moreover, plantations are designed for rapid turnover of trees, with fast growing trees harvested rapidly, undermining the role that older trees have in carbon capture and as mother trees that supporting young developing trees. As recently stated by a forestry academic, "*The value you have in large mature trees is almost incalculable, and so you should avoid losing that at any cost - regardless of how many trees you think about planting*" (BBC, 2023). Plantations such as those envisaged by the Living Carbon Project may be thought of as a dangerous distraction that can drive opportunity costs away from the essential work required to preserve and re-build natural forests. Finally, the expansion of plantations facilitates expansion into fertile agricultural land, jeopardizing human livelihoods, agrarian reform policies, land rights and sovereignty (Canadian Biotechnology Action Network and the Campaign to STOP GE Trees, 2022). The GM rubber tree in India is another example of a crop now being targeted for cultivation in plantations outside of its natural range in the

country; and raises concerns regarding potential export to other rubber plantation economies in Thailand, Indonesia, Vietnam, India, Cote d'Ivoire, mainland China, and Malaysia (Canadian Biotechnology Action Network and the Campaign to STOP GE Trees, 2022).

Evolutionary responses to trees

The consequences of transgene expression in natural ecosystems in evolutionary and ecological processes is a major concern for GMOs, and in particular long-lived organisms such as trees. When engineering at a population level, e.g., of wild forest ecosystems, evolutionary dynamics need to be considered. Evolutionary processes make it possible to turn events with a low probability of ever happening into events that may feasibly happen, and may be impossible to detected or mitigated over the time-scales of tree species. GM may influence evolutionary processes by, for example, altering the fitness of a GM tree such that it may be survive beyond the normal range of a conventional counterpart, e.g., by being able to withstand chemical pesticides. More relevant to current tree projects is the potential to alter the evolutionary processes of pathogens. Forests are under increasing threat as a result of disease and pathogen attacks (Davis, 2021), and thus ensuring forest resilience against disease is an obvious priority for forest management. Such interactions are environmentally mediated and thus very difficult to predict or assess. With the development of disease resistant trees, the potential arises to encourage the evolution of pathogen or pest resistance as has already been documented with the GM papaya (see above) as well as with commercialised GM crops. The short life spans of pathogens versus GM trees means that the evolution of resistance in pests will likely develop rapidly upon exposure to even to a single generation of GM trees.

Attempts to develop pathogen-tolerant GM trees also come with intrinsic risks of generating a reservoir of disease within populations. This risk is acknowledged by GM Chestnut developers (State University of New York College of Environmental Science and Forestry, 2020), though the risk of such a scenario has been entirely ignored in their application for release. Breeding programs, surviving populations, as well as hybrid varieties that have been introduced from Chinese varieties, may well be at risk of increased blight as a result of such a scenario. GM Chestnuts are developed to tolerate the blight fungus, such that it survives and can spread. Brewer (1995) found that the spread of blight is greater where there are greater numbers of trees that can be infected. Boland (2012) state (p.9) that, "*Locations for new plantings of American Chestnut for restoration or nut crops should be chosen carefully as they may act as a bridge to connect diseased populations of American Chestnut to isolated populations that have escaped disease*".

As discussed above, with trees designed to resist insects, such as Bt poplar trees, there is also the potential for the evolution of resistance in target pests, as well as secondary pest infestations following any suppression of target pests. Secondary pest infestations are commonly associated with Bt crops and have often led to crop failures (Sirinathsinghji, 2022). The potential for secondary pest infestations within complex forest ecosystems raises further uncertainty over ecological consequences, and may exacerbate the threats that invasive species already pose to tree species. Similarly, as discussed above, weeds evolve resistance to blanket spraying of GM crops with the associated herbicide, and this leads to increased use of herbicides and associated threats to biodiversity (GeneWatch UK, 2022). Similar effects could pose similar or greater risks to forest ecosystems.

Challenges to efficacy

There are several challenges to efficacy that severely undermine the rationale for any go-ahead of GM tree releases. These include both technical challenges as well as wider

questions over the use of reductionist approaches that aim to tackle single problems using simplistic GM traits aimed for introduction into complex ecosystems.

Technical challenges have hampered the development of products largely to those already commercialised in the agricultural sector. These commonly used traits are now widely associated with efficacy problems, e.g., the development of resistance to viruses (Papaya), insects (Bt crops) and weeds (crops with tolerance to glyphosate and other herbicides). Despite their limitations, R&D has not progressed much beyond these traits since GM tree technologies were first investigated. Trait development still overwhelmingly focuses on the profitability of industrialised wood plantation industries, yet the decades of research into altering wood properties, e.g., via lignin modification, has been dogged with efficacy problems that are a testament to the complexity and unpredictability of the GM process, particularly in complex organisms such as trees.

Thus, promises have been made for more than 20 years, that have not been delivered. For example, in 2003, Sedjo (2003) claimed that, "*Genetically modified (GM) or transgenic trees are approaching commercialization in forestry*", and concluded that, "*The economics suggest that social benefits could be obtained from lower-cost wood production that might be forthcoming from transgenic trees*". Sedjo (2005) again suggests that "*GE [genetically engineered] trees have the potential to provide substantial financial and economic returns under appropriate conditions*" and that these conditions might be particularly favourable in developing countries such as China and Brazil. However, Sedjo (2003 and 2005) was not correct in this prediction: there are still no plantation or forest GM trees on the commercial market.

Attempts to alter lignin have thus far been associated with a variety of problems including growth retardation. Lignin is a structural molecule that is essential for structural integrity as well as providing defence against pest and pathogen attacks. Pleiotropic effects of lignin alteration, where a single gene influences two or more apparently independent traits, have been associated with alterations in flower morphology and pigmentation in plants, for example (Federation of German Scientists, 2008; Tzfira et al., 1998). To date, most single gene modifications have had adverse effects on growth, or alternatively, the effects of modification have not translated to real-world alterations in field trials. For example, reduction in lignin for the purpose of biofuel production does not necessarily result in increased wood processability (Voelker et al., 2010), with reductions of lignin adversely affecting wood chemistry and plant metabolism. Likewise, a long term study in Wenling (China), found that lignin-modified poplars did not show a significant improvement in sugar yield compared to the wild type (Wang et al., 2018). Other studies that have reported lignin reductions have found that reductions disappear or lessen when trees are subject to environmental conditions versus controlled lab studies (Bryant et al., 2020). The unintended increased production of extractive molecules such as complex polyphenols deposited in wood, interfere with the process (also leading to discoloration and deformed stems). Work continues to optimise altered lignin modifications to reduce unintended effects, but this may result in trees being developed with more complex modifications with additional genes, e.g., the insertion of curcumin (derived from haldi, a.k.a. turmeric) as an alternative to lignin as means to increase biofuel processing (summarised by Bryant et al., 2020).

Significant technical bottlenecks also remain for GM trees that limit the number of species that can be modified by both first-generation and newer GM techniques such as genome editing. Most genome editing research is still dedicated to basic research. Compared to annual plants, trees are more difficult to engineer due to long vegetative periods, low transformation efficiency, difficulty in growing tissue cultures, and a limited number of mutants (Fan et al., 2015; Osakabe et al., 2016). Moreover, many economically valuable tree species currently cannot be genome edited, because they are recalcitrant to

Agrobacterium transformation and/or to tissue culture regeneration (Bewg et al., 2018). In addition, commercial production heavily relies on clonal propagation of elite genotypes (Bewg et al., 2018). The term clone is used to mean a genetically uniform plant material derived from a single individual and propagated exclusively by vegetative means (rather than by planting seed): this is an expensive, specialist process. Finally, outcrossing tree species creates additional problems in generating stable inheritance of modifications due to a high degree of heterozygosity (meaning lots of genetic variability). This makes target sites destined for modification harbour distinct sequences, and thus renders editing ineffective. To generate homozygous trees, where all copies of a gene are mutated, further requires multigenerational screening which is hampered by long generational times of trees. Moreover, while many in the GMO industry see genome editing as a way around GMO regulations (if the final product can be deemed free of transgenic material), the reality is more complicated. The vast majority of genome editing still requires the introduction of transgenic material, including transgenes encoding for the editing machinery. Engineers will often then attempt to breed out the transgenes while attempting to preserve the 'edited' change in the new GMO organism. However, in trees the low efficiency of the engineering process, along with the delayed reproduction and high levels of heterozygosity in forest trees, makes it extremely difficult to breed out any transgenic material at scale. How genome editing can really be deployed to address forest health beyond what is already being developed with standard transgenic technologies remains highly questionable. Hence, genome editing serves as a distraction to more viable forest management systems.

Other GM techniques such as the use of RNA interference (RNAi) technologies (as being deployed by the Living Carbon project, and commercialised papaya and apple varieties), are subject to unstable functioning. RNAi is a cellular process that can be hijacked by GM developers to switch off, or 'silence' genes (Jackson et al., 2003). However, unstable downregulation is a shortcoming of the technology, which has been reported for lignin-modified trees (Leplé et al., 2007; Van Acker et al., 2014b; Voelker et al., 2010). Moreover, off-target activity of the technology can also target additional genes for silencing, complicating and camouflaging the effects of the process.

The long R&D process for GM tree development is challenged at a wider efficacy level by the advances being made in conventional breeding. The GM eucalyptus approved in Brazil is an example. Developers report in the risk assessment, that the GM tree was developed from tree clones from a decade ago, though conventional breeding programs have since produced clones "*with properties that are similar to the ones of the genetically modified plant*". Ironically, this admission of the lack of improved efficacy over new conventional varieties was used as the basis of their safety claim that any increased water usage by the trees as a result of the faster growth trait would not pose a greater risk beyond conventional varieties (National Technical Biosafety Commission, 2015).

The reductionist approaches of GM trees struggle to deal with problems faced by trees living in wild ecosystems, where environmental effects can mediate, compensate or over-shadow any positive impacts of a given trait. As seen with the GM Chestnut trials, a wide range of pests damaged both the GM and non-GM seedlings (State University of New York College of Environmental Science and Forestry, 2020). Similarly, suggestions to engineer ash trees to be resistant to the fungal pathogen that causes die-back, may be of limited use when the invasive grey squirrel is a major cause of UK forest damage. Rubber plants are yet another example of a crop that is being modified to grow beyond its natural climatic range, yet global production is under threat from pathogens such as leaf blights, and white root disease. Moreover, traits such as sterility fail to take into account the characteristics of trees in question, which may affect potential efficacy. Flower sterility does not deal with potential gene flow or contamination via vegetative propagation, nor does it consider that the long-lived nature of trees raises uncertainty regarding the stability of the trait and whether it would

maintain sterility throughout its life-span. Indeed, as raised in the section on molecular impacts above, sterility traits have been shown to be unstable in experimental projects.

Regulatory considerations

Currently, GM trees are regulated in the same manner as other GM crop products, with legislation across the world largely designed for assessing risks relevant to annual crop production. Existing risk assessments across the globe already face routine challenges by those concerned over GM crop releases, for a lack of stringency in considering all potential biosafety as well as wider socio-economic and ethical considerations. For trees, current risk assessments are arguably entirely unfit for purpose to deal with all potential risks. Moreover, GMO regulations are currently being aggressively lobbied against by the GMO industry to expand and accelerate commercialisation, with some national authorities now excluding new forms of genetic engineering from legislation, including certain forms of genome editing that do not intentionally result in introducing foreign genetic material into an organism. This includes countries such as Canada and Brazil. On-going discussions are also taking place in the EU, with the potential for the region to shift towards deregulation of GMOs not designed to carry transgenic material. Currently, EU legislation would regulate any genome edited tree as a GMO.

In nations such as the US, where the vast majority of GMOs are already deregulated, certain GM tree applications may slip through without any regulatory oversight at all¹. In the US, GMOs are regulated by three authorities, the USDA, EPA and FDA. Depending on the trait, GM trees may require little, if any, oversight from these authorities. There may be no oversight unless the plant is considered a plant pest or carrying foreign DNA that is derived from a plant pest (regulated by the USDA), to possess pesticidal properties (regulated by the EPA), or may enter the food supply (voluntary consultation with the FDA). Environmental impact assessments (EIAs) may be performed under the purview of the USDA and lack sufficient environmental consideration. As of 2018, the USDA had received 6 petitions for deregulated status (5 fruit trees and a eucalyptus), though only the eucalyptus tree was subjected to an environmental risk assessment to comply with National Environmental Policy Act (NEPA).

The GM Chestnut, currently seeking a 'deregulation' decision from the USDA, has triggered an environmental impact statement (EIS) to comply with NEPA, as well as a plant pest risk assessment, though they are insufficient in assessing all potential risks. The plant pest risk assessment ignores the risks of the trees being potential reservoirs for blight. Moreover, the EIS fails to include any new evidence beyond what was submitted by the developers in their initial application for deregulation status. No studies have been done to assess potential allergenicity of pollen (only rudimentary allergenicity food safety assessments have also been performed on chestnut; which is also yet to be approved by for consumption by the

¹ Three processes are available for gaining approval for environmental release under the USDA. First, a 'notification' can be given for limited releases of plants that meet certain criteria such as containment standards, though these are virtually non-existent for trees as they do not apply to releases that last longer than a year. Second, a 'permit' can be sought for limited release, which requires a more detailed application process, and is required for field trial releases. Third, is the process of "petition for deregulated status", which is the primary path to commercialisation. However, the USDA is limited to regulating GMOs that are also deemed to be a plant pest, including those that carry inserted foreign DNA derived from a plant pest. There is no oversight from the USDA once a GMO is approved for 'deregulation'. The FDA only requires voluntary consultation on GMOs that may enter the food system, i.e., food additives; and the EPA covers only pesticides, i.e., GMOs that are designed to be insecticidal, for example.

FDA). The risk of the inclusion of antibiotic resistance has not been assessed. Most crucially, the potential contamination of native trees, that would not be monitored following deregulation, has not been sufficiently addressed, despite it being a concern repeatedly flagged by the public, and even widely acknowledged by GM tree proponents (Strauss et al., 2009) as a general risk of GM trees. In fact, the EIS states that under deregulation status, "*pollen-mediated gene flow from Darling 58 American Chestnut to wild American chestnut populations is intended*", but nonetheless that current methods used to control pollination of hybrid species can be applied. However, how successful such practices are, can be questioned, due to the lack of monitoring of such impacts (Davis, 2021). The EIS is required to analyse potential migration of the tree across borders, though this has also been ignored, with implications for restoration projects in neighbouring Canada (Canadian Chestnut Council, 2022). Impacts on non-target organisms have not been adequately addressed. The EIS cannot be relied upon to adequately deal with the risks GM trees pose to wild ecosystems, including forests.

International regulations under the Convention for Biological Diversity's (CBD) legally-binding Cartagena Protocol on Biosafety (CPB) provide minimal standards for risk assessment to signatories of the Protocol, which include countries such as Brazil who recently approved a GM eucalyptus variety. In 2008, Parties called for additional guidance to specifically deal with the risks posed by GM trees. Additional guidance has since been drawn up, in recognition of "*the uncertainties related to the potential environmental and socio-economic impacts, including long term and transboundary impacts, of genetically modified trees on global forest biological diversity*", and it has been recommended that "*Parties to take a precautionary approach when addressing the issue of genetically modified trees*" (CBD, 2016). The guidance refers to specific risks that differ from other plants, such as annual crops, due to characteristics such as size, perennial growth habit with a long lifespan, and delayed onset of reproductive maturity, as well as the various pathways for dispersal and thus contamination. However, this additional guidance is not mandated for signatories of the Protocol. The recent approval in Brazil of the GM tree suggests that such guidance was not taken on board with only standard risk assessment (RA) procedures being followed. In addition, a lack of transparency regarding confidential business information has hindered the availability of the data in the performed risk assessment. The risk assessment, for example, also failed to address again the issue of allergenicity of pollen, which was shown to express the transgenes, and which will also present in honey samples. Issues relating to ecosystem health, such as potential increased water usage, were dismissed as being equivalent to newly developed conventional varieties that have "similar properties" to the GM 'faster growing' trees, undermining the very rationale for the project while simultaneously ignoring the risk raised in a public hearing, and that the RA was purportedly aiming to address.

Consequences of potential deregulation of genome editing

GMO regulations across the world are under threat due to the deployment of genome editing techniques being promoted by GMO proponents as safe, effective and/or equivalent to conventional varieties in order to exclude them from GMO regulations. Some countries have already made decisions to deregulate either some or all forms of edited products, while other countries and regions are still deliberating. Nevertheless, the vast majority of countries do still regulate genome edited products as GMOs.

Any exclusion of genome edited products from national or regional legislation raises significant environmental, food safety, trade and economic implications. Exclusion of genome edited trees may allow for environmental releases without the requirement for biosafety risk assessments to the environment or human health. Monitoring requirements to assess potential adverse impacts of genome edited trees could also be lost, an issue that is

particularly pertinent to long living organisms being released into the wild. Removal of traceability requirements would also prevent the ability for independent monitoring, e.g., of conservation projects that aim to assess the impacts of GE tree releases for restoration purposes. With regard to food products, traceability is also essential to allow trade with countries that do have regulations in place. Moreover, traceability allows farmers, retailers and consumers to choose to grow or consume conventional varieties or organic produce without potential for contamination that may jeopardise trade and consumer choice. Finally, these regulations operationalise mechanisms to remove, or recall, organisms and their products from the environment or food markets, if problems do indeed arise.

Those lobbying for deregulation see it as a means to accelerate pathways to market by allowing products to be released without what they claim are 'costly and lengthy' regulatory requirements, such as biosafety risk assessments. However, deregulation also poses potential complications for the industry. For example, within the UK's four nations, England is currently proposing the deregulation of some gene edited products, while Scotland and Wales are proposing to regulate them. Without traceability requirements, monitoring the spread of pollen across the border may not be possible. Divergent regulations also have major trade implications, with, for example, the potential for a country to ban all imported products from a nation that does not regulate or trace gene edited organisms which would not be identifiable without labelling. GM trees that are not marketed as food products may still have trade impacts, with releases potentially inadvertently contaminating the food supply, for example, eucalyptus pollen-derived honey.

Beyond the hype, any deregulation of genome edited products under the guise of its revolutionary ability to generate needed traits, free from transgenic material, remains dubious. As raised above, the reality is that genome edited tree development remains a lengthy process, with current applicability also limited to a few species. The fact that the vast majority of editing techniques include the introduction of transgenes that have to be later removed by breeding, puts significant brakes on any rapid development of transgene-free trees. Such technical issues suggest a level of hubris common to the GMO industry that repeatedly serves to generate short term profits while distracting attention from more viable, long-term solutions. Significant opportunity costs are a risk of succumbing to the regular hype surrounding genome editing as a means to address any food or environmental issue. Moreover, free of transgenes or not, any release of a genome edited tree would still be the release of a modified organism carrying a novel combination of genetic material with both intended, as well as potentially unintended changes to the genome.²

Conclusion

GM tree development has faced a number of technical challenges that have hindered widespread production of GM tree traits. Nonetheless, a limited number of GM tree commercialisations are potentially on the horizon for release, including into wild forest

² It is worth noting that techniques are under development to allow the bypassing of transgenic processes, such as the grafting of shoots from conventional varieties onto GM rootstock that have been modified to release genome editing machinery into the grafted material (L. Yang et al., 2023). Such techniques are experimental and aim to avoid intended introduction of exogenous DNA, but this does not get around unintended DNA introductions and other observed, unintended molecular impacts of genome editing techniques. For example, even without the introduction of any DNA, exogenous DNA can still be integrated via the production of DNA copies of the guide RNA molecules that are introduced into the target organism as part of the CRISPR genome editing machinery (Petri et al., 2022).

ecosystems. Such releases may now be technically feasible, yet fundamental challenges to their potential ‘success’ and safety remain when taking into consideration the wider complexities of forest and wild ecosystems. Modifying wild ecosystems raises fundamental risks and uncertainties that cannot be adequately assessed with current GMO risk assessment processes. Current GM assessments omit analysis of forest health and potential long-term impacts on the full range of parameters that are relevant to tree releases, including impacts on soil, species-interactions, water systems, gene flow, nutrient uptake, disease resilience, stress resilience, communication networks, and evolutionary processes. But more crucially, it remains highly questionable whether any risk assessment can ever be sufficiently robust to ensure against harms, given current human understanding and lack of knowledge about trees and forest environments.

As such, promoting GM trees as emergency measures to address environmental problems currently at the forefront of people’s concerns, may merely serve to undermine much harder, ongoing work to restore forest ecosystems, including decades-long American Chestnut breeding programs. The potential for significant opportunity costs may result from any focus on GM projects with lofty goals that risk acting as little more than a distraction. Efforts to supposedly appease public anxieties over widespread environmental destruction may, instead, entrench the environmental or food problems they purport to address, if more viable alternatives are neglected.

The expansion of plantation monocultures as a result of GM commercial forestry threatens to undermine efforts to restore healthy forests and surrounding, critical ecosystems such as savannahs and peatlands. Moreover, forests themselves have been shown to have an immense capacity to regenerate. Rather than expanding plantations under the guise of climate action or restoration, natural forests should be stewarded to recover and be managed in context and knowledge specific manners that incorporate the existing forms of regeneration (e.g. Schmidt et al., 2021). Given the lifespan of trees, any GM releases could have impacts that manifest over centuries and even millennia. Although humanity can release GM trees, it remains extremely questionable whether we will ever be confident of the long-term impacts. As raised by a US EPA official in reference to the GM Chestnut, “*Just because we can do something, should we?*” (Grandoni, 2022).

Appendix 1. Summary of Global Field Trials

Below is a summary of field trials being conducted in different global regions. These summaries exemplify the limitations of GM research to date, which have suffered from a narrow focus on a few tree species and traits. Technical limitations hamper which species can be modified, and what traits can be developed. Knowledge gaps also hinder what traits can be developed with understanding of gene function still limited. Financial motivations have also served to direct R&D towards a few traits beyond those that aim to maximise industrial plantation industry profits.

Table 1

a) EU field trials conducted since 2002:

Country	Species	Trait	Application date
Sweden	Grey Poplar	Wood quality (lignin) Genome edited (CRISPR/Cas system)	2022
	Poplar (hybrid aspen)	Wood quality (lignin) Genome edited (CRISPR/Cas system)	2020
	Poplar (hybrid aspen)	Wood quality (lignin or xylan)	2018
	Poplar (hybrid aspen)	Growth alterations	2017
	Poplar (hybrid aspen)	Altered phenology	2016
	Poplar (hybrid aspen)	Wood biomass	2016
	Apple rootstock grafting	Growth alterations	2016
	Poplar (hybrid aspen)	Growth alterations	2014
	Poplar (hybrid aspen)	Wood quality (lignin)	2014
	Poplar (hybrid aspen)	Altered phenology	2012
	Poplar (hybrid aspen)	Altered growth	2011
	Poplar (hybrid aspen)	Wood quality	2011
	Poplar (hybrid aspen)	Altered growth/drought stress	2011
	Apple rootstock grafting	Rooting ability	2010
	Poplar (hybrid aspen)	Altered growth	2010

	Poplar (hybrid aspen)	Wood quality (lignin)	2008
Belgium	Grey Poplar	Wood quality	2021
	Grey Poplar	Wood quality (lignin)	2013
	Grey Poplar	Wood quality (lignin)	2009
	Apple	Altered flowering, self-compatibility, fruit quality, yield	Application rejected in 2002 based on concerns over genetic contamination
Finland	Poplar (hybrid aspen)	Wood quality	2018
	Poplar (hybrid aspen)	Wood quality	2018
	Silver Birch	Sterility/altered flowering	2018
	Poplar (hybrid aspen)	Wood quality	2013
	Silver Birch	Sterility/altered flowering	2018
	Silver Birch	Sterility/altered flowering	2018
Netherlands	Apples	Altered food quality	2015
	Apples	Fungal resistance	2015
	Apples	Altered biomass	2012
Spain	Poplar (hybrid aspen)	Altered biomass, branching	2012
	Poplar (hybrid aspen)	Altered biomass, branching	2012
	Poplar (hybrid aspen)	Altered biomass, branching	2012
France	Poplar (hybrid aspen)	Wood quality (lignin)	2013
	Poplar (hybrid aspen)	Altered wood quality for biofuels	2013
	Poplar (hybrid aspen)	Wood quality (lignin)	2007
Poland	Poplar (hybrid aspen)	Wood quality	2015
	Poplar	Altered biomass	2010

b) USA:

Species	Number of permits	Traits included	Date of permit issued
Poplar	51	19 product quality; 10 herbicide tolerance, 16 agronomic quality, 5 insect resistance	1989-2021
Apple	34	11 altered agronomic quality; 17 altered product quality	1995-2020

Chestnut	29	22 fungal resistance; 12 herbicide tolerance	1997-2021
Eucalyptus	21	2 herbicide tolerance; 7 altered wood quality; 10 sterility	2007-2019
Walnut	14	10 bacterial resistance	1990-2021
Sweetgum	6	4 sterility; 1 herbicide tolerant; 1 altered growth	1996-2020
Rubber	4	3 yield, 1 pest resistance	2007-2008
Plum	13	5 fungal resistance; 9 altered agronomic quality; 1 bacterial resistance	
Pine	8	Altered growth rate	2008-2014

Table 2 GM Trees registered in Convention for Biological Diversity

Decisions regarding the import or release of LMOs that may be subject to transboundary movement are required to be submitted to the CBD's Biosafety Clearing House. The responsibility lies with countries to submit this information. As such, some records may be incomplete.

Tree	Country and Date of registry
Herbicide-tolerant eucalyptus	Brazil, 2022
ESF-DAR58-3 - Blight-tolerant Darling 58 American Chestnut	USA, 2020
Papaya modified for bacterial resistance	Malaysia, 2019
Apple (Arctic™ Fuji) 'non-browning'	Canada, 2019
Poplar with modified lignin	Belgium, 2019
Antisense hybrid aspen modified for altered phenology (circadian rhythms)	Sweden, 2019
Hybrid aspen with modified autumn phenology (circadian rhythms)	Sweden, 2019
Apple with modified food quality 'red fleshed'	Netherlands, 2017
Apple (Arctic™ "Golden Delicious") 'non-browning'	Canada, 2015
Apple (Arctic™ "Granny Smith") 'non-browning'	Canada, 2015
Apple modified for fungal resistance	Netherlands, 2015
Eucalyptus modified for increased growth and yield	Brazil, 2015
Rubber tree modified to express Human Protamine 1	Malaysia, 2015
Rubber tree modified to express the scFv4715 antibody fragment	Malaysia, 2015
Rubber tree modified to express Human atrial natriuretic factor	Malaysia, 2015
Rubber tree modified to express a reporter gene	Malaysia 2015
Bitter orange modified for bacterial resistance	Mexico, 2015

Bitter orange modified for bacterial/fungal resistance	Mexico, 2015
Bitter orange modified for bacterial/fungal resistance	Mexico, 2015
Aspen modified for increased growth rate	Russia, 2015
White birch modified for increased growth rate	Russia, 2015
White birch modified for herbicide tolerance	Russia, 2015
Aspen modified for herbicide tolerance	Russia, 2015
Papaya modified for viral resistance	USA, 2015
Papaya modified for viral resistance	USA, 2015
Silver birch lines with potential pest or fungal disease resistance	Finland, 2015
Hybrid aspen lines with modified wood development	Finland, 2014
Hybrid aspen lines with modified stomatal regulation	Finland, 2014
Papaya modified for viral resistance	USA, 2014
ARS-PLMC5-6 - Plum Tree modified for resistance to Plum pox virus	United states, 2014
Papaya modified for delayed ripening	Malaysia, 2013
Papaya modified for viral resistance	USA, 2013
Poplar with modified lignin	Belgium, 2012
Cold tolerant eucalyptus	Japan, 2011
Apple modified for non-flowering, fungal resistance	Netherlands, 2012
Grape vine modified for resistance to fungal pathogens	2017 (authorised in 1999 prior to Cartagena protocol)
Grape vine modified for resistance to fungal pathogens	2017 (authorised in 1999 prior to Cartagena protocol)
Grape vine modified for the expression of a marker gene	2017 (authorised in 1999 prior to Cartagena protocol)
Aspen, male, modified for modified for an altered morphology and reduced growth.	Germany, 2016 (authorised in 1996 prior to Cartagena protocol)
Aspen, female, modified for modified for an altered morphology and reduced growth	Germany, 2016 (authorised in 1996 prior to Cartagena protocol)
Hybrid Aspen, female, modified for an altered morphology	Germany, 2016 (authorised in 1996 prior to Cartagena protocol)
Hybrid Aspen, female, modified for an altered morphology and reduced growth	2016 (authorised in 1996 prior to Cartagena protocol)
Hybrid aspen modified for observing horizontal gene transfer into ectomycorrhizal fungi	2016 (authorised in 1996 prior to Cartagena protocol)
Poplar modified for increased glutathione content	Germany, 2012 (authorised in 1996 prior to Cartagena protocol)

References

- Adnan, M., Khan, S., Patel, M., Al-Shammari, E., & Ashankyty, I. M. A. (2013). *Agrobacterium*. *Reviews in Medical Microbiology*, 24(4), 94–97. <https://doi.org/10.1097/MRM.0b013e3283642449>
- Alvi, A. H. K., Sayyed, A. H., Naeem, M., & Ali, M. (2012). Field Evolved Resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* Toxin Cry1Ac in Pakistan. *PLoS ONE*, 7(10), e47309. <https://doi.org/10.1371/journal.pone.0047309>
- Battaglin, W. A., Smalling, K. L., Anderson, C., Calhoun, D., Chestnut, T., & Muths, E. (2016). Potential interactions among disease, pesticides, water quality and adjacent land cover in amphibian habitats in the United States. *Science of The Total Environment*, 566–567, 320–332. <https://doi.org/10.1016/j.scitotenv.2016.05.062>
- Bauer-Panskus, A., Miyazaki, J., Kawall, K., & Then, C. (2020). Risk assessment of genetically engineered plants that can persist and propagate in the environment. *Environmental Sciences Europe*, 32(1), 32. <https://doi.org/10.1186/s12302-020-00301-0>
- Bauman, J. M., Francino, S., & Santas, A. (2018). Interactions between ectomycorrhizal fungi and chestnut blight (*Cryphonectria parasitica*) on American chestnut (*Castanea dentata*) used in coal mine restoration. *AIMS Microbiology*, 4(1), 104–122. <https://doi.org/10.3934/microbiol.2018.1.104>
- Bayle, G. (2019). Ecological and social impacts of eucalyptus tree plantation on the environment. *Journal of Biodiversity Conservation and Bioresource Management*, 5(1), 93–104. <https://doi.org/10.3329/jbcbm.v5i1.42189>
- BBC (Director). (2022, August 25). Are genetically modified trees the secret to capturing our planet's excess carbon? In *Positive Thinking*. <https://www.bbc.co.uk/sounds/play/m001bbwc?partner=uk.co.bbc&origin=share-mobile>
- BBC. (2023). UK's old trees critical to climate change fight. *BBC*. <https://www.bbc.co.uk/news/science-environment-64028694>
- Beckers, B., Op De Beeck, M., Weyens, N., Boerjan, W., & Vangronsveld, J. (2017). Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome*, 5(1), 25. <https://doi.org/10.1186/s40168-017-0241-2>
- Beugnon, R., Ladouceur, E., Sünnemann, M., Cesarz, S., & Eisenhauer, N. (2022). Diverse forests are cool: Promoting diverse forests to mitigate carbon emissions and climate change. *Journal of Sustainable Agriculture and Environment*, 1(1), 5–8. <https://doi.org/10.1002/sae2.12005>
- Bewg, W. P., Ci, D., & Tsai, C.-J. (2018). Genome Editing in Trees: From Multiple Repair Pathways to Long-Term Stability. *Frontiers in Plant Science*, 9. <https://doi.org/10.3389/fpls.2018.01732>
- Boland, G. J. (2012). *American Chestnut (Castanea dentata) in Ontario*. Ministry of Natural Resources.
- Bondera, M., & Query, M. (2006). *Hawaiian papaya: GMO contaminated*. Hawaii SEED. <https://hawaiiseed.org/wp-content/uploads/2012/11/Papaya-Contamination-Report.pdf>
- Branford, S. (2021). Brazil's Suzano boasts its pulpwood plantations are green; critics disagree. *Mongabay*. <https://news.mongabay.com/2021/12/brazils-suzano-boasts-its-pulpwood-plantations-are-green-critics-disagree/>
- Brewer, L. G. (1995). Ecology of Survival and Recovery from Blight in American Chestnut Trees (*Castanea dentata* (Marsh.) Borkh.) in Michigan. *Bulletin of the Torrey Botanical Club*, 122(1), 40. <https://doi.org/10.2307/2996402>
- Brockhoff, E. G., Barbaro, L., Castagneyrol, B., Forrester, D. I., Gardiner, B., González-Olabarria, J. R., Lyver, P. O., Meurisse, N., Oxbrough, A., Taki, H., Thompson, I. D.,

- van der Plas, F., & Jactel, H. (2017). Forest biodiversity, ecosystem functioning and the provision of ecosystem services. *Biodiversity and Conservation*, 26(13), 3005–3035. <https://doi.org/10.1007/s10531-017-1453-2>
- Bryant, N. D., Pu, Y., Tschaplinski, T. J., Tuskan, G. A., Muchero, W., Kalluri, U. C., Yoo, C. G., & Ragauskas, A. J. (2020). Transgenic Poplar Designed for Biofuels. *Trends in Plant Science*, 25(9), 881–896. <https://doi.org/10.1016/j.tplants.2020.03.008>
- Canadian Chestnut Council. (2022). *Comments to the United States Department of Agriculture regarding The State University of New York College of Environmental Studies and Forestry Petition (19-309-01p) for Determination of Nonregulated Status for Blight-Tolerant Darling 58 American Chestnut Draft Environmental Impact Statement & Draft Plant Pest Risk Assessment*. <https://www.regulations.gov/comment/APHIS-2020-0030-11639>
- CBD. (2016). *GUIDANCE ON RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS AND MONITORING IN THE CONTEXT OF RISK ASSESSMENT. UNEP/CBD/BS/COP-MOP/8/8/Add.1*. <https://www.cbd.int/doc/meetings/bs/mop-08/official/bs-mop-08-08-add1-en.pdf>
- Chanoca, A., de Vries, L., & Boerjan, W. (2019). Lignin Engineering in Forest Trees. *Frontiers in Plant Science*, 10, 912. <https://doi.org/10.3389/fpls.2019.00912>
- Chen, X.-H., Wang, F.-L., Zhang, R., Ji, L.-L., Yang, Z.-L., Lin, H., & Zhao, B. (2016). Evidences of inhibited arbuscular mycorrhizal fungal development and colonization in multiple lines of Bt cotton. *Agriculture, Ecosystems & Environment*, 230, 169–176. <https://doi.org/10.1016/j.agee.2016.05.008>
- Clark, S. L., Schlarbaum, S. E., Pinchot, C. C., Anagnostakis, S. L., Saunders, M. R., Thomas-Van Gundy, M., Schaberg, P., McKenna, J., Bard, J. F., Berrang, P. C., Casey, D. M., Casey, C. E., Crane, B., Jackson, B. D., Kochenderfer, J. D., Lewis, R. F., MacFarlane, R., Makowski, R., Miller, M. D., ... Williamson, T. S. (2014). Reintroduction of American Chestnut in the National Forest System. *Journal of Forestry*, 112(5), 502–512. <https://doi.org/10.5849/jof.13-106>
- Committee on the Potential for Biotechnology to Address Forest Health, Board on Agriculture and Natural Resources, Division on Earth and Life Studies, & National Academies of Sciences, Engineering, and Medicine. (2019). *Forest Health and Biotechnology: Possibilities and Considerations* (p. 25221). National Academies Press. <https://doi.org/10.17226/25221>
- Davidson, S. N. (2008). Forbidden fruit: Transgenic papaya in Thailand. *Plant Physiology*, 147(2), 487–493. <https://doi.org/10.1104/pp.108.116913>
- Davis, D. E. (2021). *The American chestnut: An environmental history*. The University of Georgia Press.
- Defarge, N., Otto, M., & Hilbeck, A. (2023). A Roundup herbicide causes high mortality and impairs development of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). *Science of The Total Environment*, 865, 161158. <https://doi.org/10.1016/j.scitotenv.2022.161158>
- Dhurua, S., & Gujar, G. T. (2011). Field-evolved resistance to *Bt* toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India: Resistance of pink bollworm, *Pectinophora gossypiella* (Saunders), to *Bt* toxin Cry1Ac. *Pest Management Science*, 67(8), 898–903. <https://doi.org/10.1002/ps.2127>
- El-Lakany, M. (2004). Are genetically modified trees a threat to forests? *Unasylva*, 55(217), 45–47.
- ENSSER. (2021). *SCIENTIFIC CRITIQUE OF LEOPOLDINA AND EASAC STATEMENTS ON GENOME EDITED PLANTS IN THE EU*. <https://ensser.org/wp-content/uploads/2021/04/Greens-EFA-GMO-Study-1.pdf>
- Fan, D., Liu, T., Li, C., Jiao, B., Li, S., Hou, Y., & Luo, K. (2015). Efficient CRISPR/Cas9-mediated Targeted Mutagenesis in *Populus* in the First Generation. *Scientific Reports*, 5. <https://doi.org/10.1038/srep12217>
- FAO. (2004). *Preliminary review of biotechnology in forestry, including genetic modification* (Forest Genetic Resources Working Paper FGR/59E). Forest Resources

- Development Service, Forest Resources Division. Rome, Italy.
<https://www.fao.org/3/ae574e/ae574e00.htm>
- Faruk, A., Belabut, D., Ahmad, N., Knell, R. J., & Garner, T. W. J. (2013). Effects of Oil-Palm Plantations on Diversity of Tropical Anurans. *Conservation Biology*, 27(3), 615–624.
<https://doi.org/10.1111/cobi.12062>
- Fatoretto, J. C., Michel, A. P., Silva Filho, M. C., & Silva, N. (2017). Adaptive Potential of Fall Armyworm (Lepidoptera: Noctuidae) Limits Bt Trait Durability in Brazil. *Journal of Integrated Pest Management*, 8(1). <https://doi.org/10.1093/jipm/pmx011>
- Federation of German Scientists. (2008). *Genetically Engineered Trees & Risk Assessment. An overview of risk assessment and risk management issues*. Federation of German Scientists. https://www.econexus.info/files/GE-Tree_FGS_2008.pdf
- Fillatti, J. J., Sellmer, J., McCown, B., Haissig, B., & Comai, L. (1987). Agrobacterium mediated transformation and regeneration of Populus. *Molecular and General Genetics MGG*, 206(2), 192–199. <https://doi.org/10.1007/BF00333574>
- FirstFruits. (n.d.). <https://firstfruits.com>.
- Flores, S., Saxena, D., & Stotzky, G. (2005). Transgenic Bt plants decompose less in soil than non-Bt plants. *Soil Biology and Biochemistry*, 37(6), 1073–1082.
<https://doi.org/10.1016/j.soilbio.2004.11.006>
- Fork, S., Woolfolk, A., Akhavan, A., Van Dyke, E., Murphy, S., Candiloro, B., Newberry, T., Schreibman, S., Salisbury, J., & Wasson, K. (2015). Biodiversity effects and rates of spread of nonnative eucalypt woodlands in central California. *Ecological Applications*, 25(8), 2306–2319. <https://doi.org/10.1890/14-1943.1>
- Frankenhuyzen, K. van, & Beardmore, T. (2004). Current status and environmental impact of transgenic forest trees. *Canadian Journal of Forest Research*, 34(6), 1163–1180.
<https://doi.org/10.1139/x04-024>
- Frontier. (2022). *Frontier facilitates first carbon removal purchases*.
<https://frontierclimate.com/writing/spring-2022-purchases>
- Gassmann, A. (2021). Resistance to Bt Maize by Western Corn Rootworm: Effects of Pest Biology, the Pest–Crop Interaction and the Agricultural Landscape on Resistance. *Insects*, 12(2), 136. <https://doi.org/10.3390/insects12020136>
- Gassmann, A. J. (2016). Resistance to Bt maize by western corn rootworm: Insights from the laboratory and the field. *Current Opinion in Insect Science*, 15, 111–115.
<https://doi.org/10.1016/j.cois.2016.04.001>
- Gassmann, A. J., Petzold-Maxwell, J. L., Keweshan, R. S., & Dunbar, M. W. (2011). Field-Evolved Resistance to Bt Maize by Western Corn Rootworm. *PLoS ONE*, 6(7), e22629. <https://doi.org/10.1371/journal.pone.0022629>
- GeneWatch UK. (2020). *GeneWatch UK submission to USDA APHIS docket APHIS-2020-0030: Petition for Determination of Nonregulated Status for Blight-Tolerant Darling 58 American Chestnut (Castanea dentata)*. GeneWatch UK.
<http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/genewatch-uk-aphis-ge-chestnut-fin.pdf>
- GeneWatch UK. (2021). *On-target effects of genome editing techniques: (Un)repaired DNA damage, a hindrance to safety and development?* GeneWatch UK.
<http://genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/genome-editing-techniques-fin.pdf>
- GeneWatch UK. (2022). *Time for the end of GM/GE herbicide tolerant crops?* GeneWatch UK. <http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/ht-report-fin.pdf>
- Glor, R. E., Flecker, A. S., Benard, M. F., & Power, A. G. (2001). [No title found]. *Biodiversity and Conservation*, 10(5), 711–723. <https://doi.org/10.1023/A:1016665011087>
- GMWatch. (2004). *More on GM papaya scandal—Government probe / NGOs may sue*. <https://gmwatch.org/en/news/archive/2004/7728-more-on-gm-papaya-scandal-government-probe-ngos-may-sue-982004>

- Gonsalves, D. (2016). The 'Rainbow' papaya experience. *Acta Horticulturae*, 1124, 11–18. <https://doi.org/10.17660/ActaHortic.2016.1124.2>
- Grandoni, D. (2022, August 30). Gene editing could revive a nearly lost tree. Not everyone is on board. *The Washington Post*. <https://www.washingtonpost.com/climate-solutions/2022/08/30/american-chestnut-blight-gene-editing/>
- Greenfield, P. (2023). Revealed: More than 90% of rainforest carbon offsets by biggest provider are worthless, analysis shows. *The Guardian*. <https://www.theguardian.com/environment/2023/jan/18/revealed-forest-carbon-offsets-biggest-provider-worthless-verra-aoe>
- Gunning, R. V., Dang, H. T., Kemp, F. C., Nicholson, I. C., & Moores, G. D. (2005). New resistance mechanism in *Helicoverpa armigera* threatens transgenic crops expressing *Bacillus thuringiensis* Cry1Ac toxin. *Applied and Environmental Microbiology*, 71(5), 2558–2563. <https://doi.org/10.1128/AEM.71.5.2558-2563.2005>
- Gutierrez-Moreno, R., Mota-Sanchez, D., Blanco, C. A., Chandrasena, D., Difonzo, C., Conner, J., Head, G., Berman, K., & Wise, J. (2020). Susceptibility of Fall Armyworms (*Spodoptera frugiperda* J.E.) from Mexico and Puerto Rico to Bt Proteins. *Insects*, 11(12), E831. <https://doi.org/10.3390/insects11120831>
- Hagenbucher, S., Wäckers, F. L., Wettstein, F. E., Olson, D. M., Ruberson, J. R., & Romeis, J. (2013). Pest trade-offs in technology: Reduced damage by caterpillars in Bt cotton benefits aphids. *Proceedings of the Royal Society B: Biological Sciences*, 280(1758), 20130042. <https://doi.org/10.1098/rspb.2013.0042>
- Hilbeck, A., Nicolas Defarge, Lebrecht, T., & Bøhn, T. (2020). *Insecticidal Bt Crops—EFSA's Risk Assessment Approach for GM Bt Plants Fails by Design*. [(Accessed on 17 December 2021)]. Available online: <https://www.testbiotech.org/en/content/rages-subreport-insecticidal-bt-crops>. https://www.testbiotech.org/sites/default/files/RAGES_report-Insecticidal%20Bt%20plants.pdf
- Hjältén, J., & Axelsson, E. P. (2015). GM trees with increased resistance to herbivores: Trait efficiency and their potential to promote tree growth. *Frontiers in Plant Science*, 6. <https://doi.org/10.3389/fpls.2015.00279>
- ISAAA. (2017). *Pocket K No. 50: Biotech/GM Trees*. <https://www.isaaa.org/resources/publications/pocketk/50/default.asp>
- Isik, F., Kumar, S., Martínez-García, P. J., Iwata, H., & Yamamoto, T. (2015). Acceleration of Forest and Fruit Tree Domestication by Genomic Selection. In *Advances in Botanical Research* (Vol. 74, pp. 93–124). Elsevier. <https://doi.org/10.1016/bs.abr.2015.05.002>
- Jackson, A. L., Bartz, S. R., Schelter, J., Kobayashi, S. V., Burchard, J., Mao, M., Li, B., Cavet, G., & Linsley, P. S. (2003). Expression profiling reveals off-target gene regulation by RNAi. *Nature Biotechnology*, 21(6), 635–637. <https://doi.org/10.1038/nbt831>
- Jain, R. K., Sharma, J., Sivakumar, A. S., Sharma, P. K., Byadgi, A. S., Verma, A. K., & Varma, A. (2004). Variability in the coat protein gene of Papaya ringspot virus isolates from multiple locations in India. *Archives of Virology*, 149(12), 2435–2442. <https://doi.org/10.1007/s00705-004-0392-x>
- Jiménez-Ramírez, A., Grivet, D., & Robledo-Arnuncio, J. J. (2021). Measuring recent effective gene flow among large populations in *Pinus sylvestris*: Local pollen shedding does not preclude substantial long-distance pollen immigration. *PLoS One*, 16(8), e0255776. <https://doi.org/10.1371/journal.pone.0255776>
- Klocko, A. L., Goddard, A. L., Jacobson, J. R., Magnuson, A. C., & Strauss, S. H. (2021). RNAi Suppression of LEAFY Gives Stable Floral Sterility, and Reduced Growth Rate and Leaf Size, in Field-Grown Poplars. *Plants (Basel, Switzerland)*, 10(8), 1594. <https://doi.org/10.3390/plants10081594>
- Kranthi, K. R. (2014). Cotton production systems—Need for a change in India. *Cotton Statistics*, 38, 4–7.

- Kunik, T., Tzfira, T., Kapulnik, Y., Gafni, Y., Dingwall, C., & Citovsky, V. (2001). Genetic transformation of HeLa cells by *Agrobacterium*. *Proceedings of the National Academy of Sciences*, 98(4), 1871–1876. <https://doi.org/10.1073/pnas.98.4.1871>
- Lebedev, V., Lebedeva, T., Tikhonova, E., & Shestibratov, K. (2022). Assessing Impacts of Transgenic Plants on Soil Using Functional Indicators: Twenty Years of Research and Perspectives. *Plants*, 11(18), 2439. <https://doi.org/10.3390/plants11182439>
- Ledford, H. (2019). Transgenic trees face rocky path from farm to forest. *Nature*, d41586-019-00072–00076. <https://doi.org/10.1038/d41586-019-00072-6>
- Leplé, J.-C., Dauwe, R., Morreel, K., Storme, V., Lapierre, C., Pollet, B., Naumann, A., Kang, K.-Y., Kim, H., Ruel, K., Lefèbvre, A., Joseleau, J.-P., Grima-Pettenati, J., De Rycke, R., Andersson-Gunnerås, S., Erban, A., Fehrle, I., Petit-Conil, M., Kopka, J., ... Boerjan, W. (2007). Downregulation of Cinnamoyl-Coenzyme A Reductase in Poplar: Multiple-Level Phenotyping Reveals Effects on Cell Wall Polymer Metabolism and Structure. *The Plant Cell*, 19(11), 3669–3691. <https://doi.org/10.1105/tpc.107.054148>
- Lim, L. C., & Traavik, T. (Eds.). (2007). *Biosafety first: Holistic approaches to risk and uncertainty in genetic engineering and genetically modified organisms*. Tapir Academic Press.
- Lindenmayer, D. B., & Hobbs, R. J. (2004). Fauna conservation in Australian plantation forests – a review. *Biological Conservation*, 119(2), 151–168. <https://doi.org/10.1016/j.biocon.2003.10.028>
- Lisch, D. (2013). How important are transposons for plant evolution? *Nature Reviews Genetics*, 14(1), 49–61. <https://doi.org/10.1038/nrg3374>
- Liu, J., Liang, Y., Hu, T., Zeng, H., Gao, R., Wang, L., & Xiao, Y. (2021). Environmental fate of Bt proteins in soil: Transport, adsorption/desorption and degradation. *Ecotoxicology and Environmental Safety*, 226, 112805. <https://doi.org/10.1016/j.ecoenv.2021.112805>
- Living Carbon Team, Tao, Y., Chiu, L.-W., Hoyle, J. W., Du, J., Rasmussen, K., Mellor, P., Richey, C., Kuiper, J., Fried, M., Dewhurst, R. A., Tucker, D., Crites, A., Orr, G. A., Heckert, M. J., Vidal, D. G., Orosco-Cardenas, M. L., & Hall, M. E. (2022). *Enhanced photosynthetic efficiency for increased carbon assimilation and woody biomass production in hybrid poplar INRA 717-1B4* [Preprint]. *Plant Biology*. <https://doi.org/10.1101/2022.02.16.480797>
- Lu, Y., Wu, K., Jiang, Y., Xia, B., Li, P., Feng, H., Wyckhuys, K. A. G., & Guo, Y. (2010). Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science (New York, N.Y.)*, 328(5982), 1151–1154. <https://doi.org/10.1126/science.1187881>
- Ludwick, D. C., Meihls, L. N., Ostlie, K. R., Potter, B. D., French, L., & Hibbard, B. E. (2017). Minnesota field population of western corn rootworm (Coleoptera: Chrysomelidae) shows incomplete resistance to Cry34Ab1/Cry35Ab1 and Cry3Bb1. *Journal of Applied Entomology*, 141(1–2), 28–40. <https://doi.org/10.1111/jen.12377>
- Mackenzie. (n.d.). *An Explanatory Guide to the Cartagena Protocol on Biosafety*.
- Mas, L. I., Aparicio, V. C., De Gerónimo, E., & Costa, J. L. (2020). Pesticides in water sources used for human consumption in the semiarid region of Argentina. *SN Applied Sciences*, 2(4), 691. <https://doi.org/10.1007/s42452-020-2513-x>
- Mo, C., Wu, Z., Xie, H., Zhang, S., & Li, H. (2020). Genetic diversity analysis of papaya leaf distortion mosaic virus isolates infecting transgenic papaya “Huanong No. 1” in South China. *Ecology and Evolution*, 10(20), 11671–11683. <https://doi.org/10.1002/ece3.6800>
- Nagrare, V. S., Kranthi, S., Biradar, V. K., Zade, N. N., Sangode, V., Kakde, G., Shukla, R. M., Shivare, D., Khadi, B. M., & Kranthi, K. R. (2009). Widespread infestation of the exotic mealybug species, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae), on cotton in India. *Bulletin of Entomological Research*, 99(5), 537–541. <https://doi.org/10.1017/S0007485308006573>

- Nair, R. J., & Bhardwaj, M. (2015, November 6). After pest attack, some Indian farmers shun GM cotton. *Reuters*. <https://www.reuters.com/article/india-cotton-gm-cotton-punjab-idINKCN0SV15T20151106>
- National Technical Biosafety Commission. (2015). *Commercial Release of genetically modified eucalyptus – Event H421*. <https://bch.cbd.int/en/database/RA/BCH-RA-BR-109752/1>
- New Scientist. (2004). China's GM trees get lost in bureaucracy. *New Scientist*. <https://www.newscientist.com/article/dn6402-chinas-gm-trees-get-lost-in-bureaucracy/>
- Osakabe, Y., Sugano, S. S., & Osakabe, K. (2016). Genome engineering of woody plants: Past, present and future. *Journal of Wood Science*, 62(3), 217–225. <https://doi.org/10.1007/s10086-016-1548-5>
- Peeters, A. G., & Zoller, H. (1988). Long range transport of *Castanea sativa* pollen. *Grana*, 27(3), 203–207. <https://doi.org/10.1080/00173138809428927>
- Petit, R. J., & Larue, C. (2022). Confirmation that chestnuts are insect-pollinated. *Botany Letters*, 169(3), 370–374. <https://doi.org/10.1080/23818107.2022.2088612>
- Petri, K., Zhang, W., Ma, J., Schmidts, A., Lee, H., Horng, J. E., Kim, D. Y., Kurt, I. C., Clement, K., Hsu, J. Y., Pinello, L., Maus, M. V., Joung, J. K., & Yeh, J.-R. J. (2022). CRISPR prime editing with ribonucleoprotein complexes in zebrafish and primary human cells. *Nature Biotechnology*, 40(2), 189–193. <https://doi.org/10.1038/s41587-021-00901-y>
- Prasad, R., & Shivay, Y. S. (2017). Oxalic Acid/Oxalates in Plants: From Self-Defence to Phytoremediation. *Current Science*, 112(08), 1665. <https://doi.org/10.18520/cs/v112/i08/1665-1667>
- Price, B., & Cotter, J. (2014). The GM Contamination Register: A review of recorded contamination incidents associated with genetically modified organisms (GMOs), 1997–2013. *International Journal of Food Contamination*, 1(1), 5. <https://doi.org/10.1186/s40550-014-0005-8>
- Rendon-von Osten, J., & Dzul-Caamal, R. (2017). Glyphosate Residues in Groundwater, Drinking Water and Urine of Subsistence Farmers from Intensive Agriculture Localities: A Survey in Hopelchén, Campeche, Mexico. *International Journal of Environmental Research and Public Health*, 14(6), 595. <https://doi.org/10.3390/ijerph14060595>
- Ryan, T. (2017). *How Trees Communicate*. National Bioneers Conference. <https://bioneers.org/teresa-ryan-bioneers-2017/>
- Schmidt, M. V. C., Ikpeng, Y. U., Kayabi, T., Sanches, R. A., Ono, K. Y., & Adams, C. (2021). Indigenous Knowledge and Forest Succession Management in the Brazilian Amazon: Contributions to Reforestation of Degraded Areas. *Frontiers in Forests and Global Change*, 4, 605925. <https://doi.org/10.3389/ffgc.2021.605925>
- Sedjo, R. (2003). Biotech and Planted Trees: Some Economic and Regulatory Issues. *AgBioForum*, 6(3), 113–119.
- Sedjo, R. (2005). Will Developing Countries be the Early Adopters of Genetically Engineered Forests? *AgBioForum*, 8(4), 205–212.
- Canadian Biotechnology Action Network and the Campaign to STOP GE Trees (2022). *The Global Status of Genetically Engineered Tree Development. A Growing Threat*. Canadian Biotechnology Action Network. <https://stopgetrees.org/wp-content/uploads/2022/09/The-Global-Status-of-Genetically-Engineered-Tree-Development-EN.pdf>
- Simard, S. (2021). *Finding the mother tree: Discovering the wisdom of the forest* (First edition). Alfred A. Knopf.
- Sirinathsinghji, E. (2022). *Bt Crops Past Their Sell-By Date: A Failing Technology Searching for New Markets?* (TWN Biotechnology & Biosafety Series 19). Third World Network and GeneWatch UK. <https://www.twn.my/title2/biosafety/bio19.htm>
- State University of New York College of Environmental Science and Forestry. (2020). *Petition for Determination of Nonregulated status for blight- tolerant Darling 58*

- American Chestnut*. <https://www.regulations.gov/document?D=APHIS-2020-0030-0002>
- Strauss, S. H., Tan, H., Boerjan, W., & Sedjo, R. (2009). Strangled at birth? Forest biotech and the Convention on Biological Diversity. *Nature Biotechnology*, 27(6), 519–527. <https://doi.org/10.1038/nbt0609-519>
- Tabashnik, B. E., & Carrière, Y. (2017). Surge in insect resistance to transgenic crops and prospects for sustainability. *Nature Biotechnology*, 35(10), 926–935. <https://doi.org/10.1038/nbt.3974>
- Tennant, P. F. (1994). Differential Protection Against Papaya Ringspot Virus Isolates in Coat Protein Gene Transgenic Papaya and Classically Cross-Protected Papaya. *Phytopathology*, 84(11), 1359. <https://doi.org/10.1094/Phyto-84-1359>
- The Hindu. (2021). First-ever genetically modified rubber planted in Assam. *The Hindu*. <https://www.thehindu.com/news/national/other-states/first-ever-genetically-modified-rubber-planted-in-assam/article34901294.ece>
- TWN. (2006). *CBD Calls For Precautionary Approach to GE Trees*. <https://biosafety-info.net/bis/agriculture-organisms-bis/cbd-calls-for-precautionary-approach-to-ge-trees/>
- Tzfira, T., Zuker, A., & Altman, A. (1998). Forest-tree biotechnology: Genetic transformation and its application to future forests. *Trends in Biotechnology*, 16(10), 439–446. [https://doi.org/10.1016/S0167-7799\(98\)01223-2](https://doi.org/10.1016/S0167-7799(98)01223-2)
- Van Acker, R., Leplé, J.-C., Aerts, D., Storme, V., Goeminne, G., Ivens, B., Légée, F., Lapierre, C., Piens, K., Van Montagu, M. C. E., Santoro, N., Foster, C. E., Ralph, J., Soetaert, W., Pilate, G., & Boerjan, W. (2014a). Improved saccharification and ethanol yield from field-grown transgenic poplar deficient in cinnamoyl-CoA reductase. *Proceedings of the National Academy of Sciences*, 111(2), 845–850. <https://doi.org/10.1073/pnas.1321673111>
- Van Acker, R., Leplé, J.-C., Aerts, D., Storme, V., Goeminne, G., Ivens, B., Légée, F., Lapierre, C., Piens, K., Van Montagu, M. C. E., Santoro, N., Foster, C. E., Ralph, J., Soetaert, W., Pilate, G., & Boerjan, W. (2014b). Improved saccharification and ethanol yield from field-grown transgenic poplar deficient in cinnamoyl-CoA reductase. *Proceedings of the National Academy of Sciences*, 111(2), 845–850. <https://doi.org/10.1073/pnas.1321673111>
- Vázquez-Barrios, V., Boege, K., Sosa-Fuentes, T. G., Rojas, P., & Wegier, A. (2021). Ongoing ecological and evolutionary consequences by the presence of transgenes in a wild cotton population. *Scientific Reports*, 11(1), 1959. <https://doi.org/10.1038/s41598-021-81567-z>
- Voelker, S. L., Lachenbruch, B., Meinzer, F. C., Jourdes, M., Ki, C., Patten, A. M., Davin, L. B., Lewis, N. G., Tuskan, G. A., Gunter, L., Decker, S. R., Selig, M. J., Sykes, R., Himmel, M. E., Kitin, P., Shevchenko, O., & Strauss, S. H. (2010). Antisense Down-Regulation of 4CL Expression Alters Lignification, Tree Growth, and Saccharification Potential of Field-Grown Poplar. *Plant Physiology*, 154(2), 874–886. <https://doi.org/10.1104/pp.110.159269>
- Wan, P., Huang, Y., Wu, H., Huang, M., Cong, S., Tabashnik, B. E., & Wu, K. (2012). Increased Frequency of Pink Bollworm Resistance to Bt Toxin Cry1Ac in China. *PLoS ONE*, 7(1), e29975. <https://doi.org/10.1371/journal.pone.0029975>
- Wang, G., Dong, Y., Liu, X., Yao, G., Yu, X., & Yang, M. (2018). The Current Status and Development of Insect-Resistant Genetically Engineered Poplar in China. *Frontiers in Plant Science*, 9, 1408. <https://doi.org/10.3389/fpls.2018.01408>
- Wang, J. P., Matthews, M. L., Williams, C. M., Shi, R., Yang, C., Tunlaya-Anukit, S., Chen, H.-C., Li, Q., Liu, J., Lin, C.-Y., Naik, P., Sun, Y.-H., Loziuk, P. L., Yeh, T.-F., Kim, H., Gjersing, E., Shollenberger, T., Shuford, C. M., Song, J., ... Chiang, V. L. (2018). Improving wood properties for wood utilization through multi-omics integration in lignin biosynthesis. *Nature Communications*, 9(1), 1579. <https://doi.org/10.1038/s41467-018-03863-z>

- Wang, S., Just, D. R., & Andersen, P. P. (2008). Bt-cotton and secondary pests. *International Journal of Biotechnology*, 10(2/3), 113. <https://doi.org/10.1504/IJBT.2008.018348>
- Williams, C. G. (2010). Long-distance pine pollen still germinates after meso-scale dispersal. *American Journal of Botany*, 97(5), 846–855. <https://doi.org/10.3732/ajb.0900255>
- Wilson, A. K. (2021). Will gene-edited and other GM crops fail sustainable food systems? In *Rethinking Food and Agriculture* (pp. 247–284). Elsevier. <https://doi.org/10.1016/B978-0-12-816410-5.00013-X>
- Wong-Bajracharya, J., Singan, V. R., Monti, R., Plett, K. L., Ng, V., Grigoriev, I. V., Martin, F. M., Anderson, I. C., & Plett, J. M. (2022). The ectomycorrhizal fungus *Pisolithus microcarpus* encodes a microRNA involved in cross-kingdom gene silencing during symbiosis. *Proceedings of the National Academy of Sciences*, 119(3), e2103527119. <https://doi.org/10.1073/pnas.2103527119>
- Woodcock, P., Cottrell, J. E., Buggs, R. J. A., & Quine, C. P. (2018). Mitigating pest and pathogen impacts using resistant trees: A framework and overview to inform development and deployment in Europe and North America. *Forestry: An International Journal of Forest Research*, 91(1), 1–16. <https://doi.org/10.1093/forestry/cpx031>
- Wu, Z., Mo, C., Zhang, S., & Li, H. (2018). Characterization of Papaya ringspot virus isolates infecting transgenic papaya ‘Huanong No.1’ in South China. *Scientific Reports*, 8(1), 8206. <https://doi.org/10.1038/s41598-018-26596-x>
- Yang, L., Machin, F., Wang, S., Saplaoura, E., & Kragler, F. (2023). Heritable transgene-free genome editing in plants by grafting of wild-type shoots to transgenic donor rootstocks. *Nature Biotechnology*. <https://doi.org/10.1038/s41587-022-01585-8>
- Yang, P., Iles, M., Yan, S., & Jolliffe, F. (2005). Farmers’ knowledge, perceptions and practices in transgenic Bt cotton in small producer systems in Northern China. *Crop Protection*, 24(3), 229–239. <https://doi.org/10.1016/j.cropro.2004.07.012>
- Yue, J., VanBuren, R., Liu, J., Fang, J., Zhang, X., Liao, Z., Wai, C. M., Xu, X., Chen, S., Zhang, S., Ma, X., Ma, Y., Yu, H., Lin, J., Zhou, P., Huang, Y., Deng, B., Deng, F., Zhao, X., ... Ming, R. (2022). SunUp and Sunset genomes revealed impact of particle bombardment mediated transformation and domestication history in papaya. *Nature Genetics*, 54(5), 715–724. <https://doi.org/10.1038/s41588-022-01068-1>
- Zhang, C. (2015). Biosafety assessment of genetically modified foods based on the toxicology of the chiral D-amino acid. *Scientia Sinica Chimica*, 45(1), 98–108.
- Zhao, G., Yan, P., Shen, W., Tuo, D., Li, X., & Zhou, P. (2015). Complete Genome Sequence of Papaya Ringspot Virus Isolated from Genetically Modified Papaya in Hainan Island, China. *Genome Announcements*, 3(5), e01056-15. <https://doi.org/10.1128/genomeA.01056-15>
- Zhu, Y. C., Blanco, C. A., Portilla, M., Adamczyk, J., Luttrell, R., & Huang, F. (2015). Evidence of multiple/cross resistance to Bt and organophosphate insecticides in Puerto Rico population of the fall armyworm, *Spodoptera frugiperda*. *Pesticide Biochemistry and Physiology*, 122, 15–21. <https://doi.org/10.1016/j.pestbp.2015.01.007>
- Zurita, G. A., Rey, N., Varela, D. M., Villagra, M., & Bellocq, M. I. (2006). Conversion of the Atlantic Forest into native and exotic tree plantations: Effects on bird communities from the local and regional perspectives. *Forest Ecology and Management*, 235(1–3), 164–173. <https://doi.org/10.1016/j.foreco.2006.08.009>
- Zwahlen, C., Hilbeck, A., Howald, R., & Nentwig, W. (2003). Effects of transgenic Bt corn litter on the earthworm *Lumbricus terrestris*. *Molecular Ecology*, 12(4), 1077–1086. <https://doi.org/10.1046/j.1365-294X.2003.01799.x>

