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Biotechnology and Genomic Approaches to Mitigating Disease Impacts on Forest Health

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Abstract

Outbreaks of insects and diseases are part of the natural disturbance regime of all forests. However, introduced pathogens have had outsized impacts on many dominant forest tree species over the past century. Mitigating these impacts and restoring these species are dilemmas of the modern era. Here, we review the ecological and economic impact of introduced pathogens, focusing on examples in North America. We then synthesize the successes and challenges of past biotechnological approaches and discuss the integration of genomics and biotechnology to help mitigate the effects of past and future pathogen invasions. These questions are considered in the context of the transgenic American chestnut, which is the most comprehensive example to date of how biotechnological tools have been used to address the impacts of introduced pathogens on naïve forest ecosystems.

1. FRAMING THE PROBLEM

Biotic disturbances, in the form of insect and disease outbreaks, are an essential part of all forest ecosystems. However, the frequency and severity of outbreaks have increased since the middle of the nineteenth century (4, 11, 100). The most recent national assessment of insect and disease impacts in the United States estimated that 32.9 million hectares of wildland forest were at risk of losing at least 25% of the tree cover between 2013 and 2027 (114). This was an increase of 9.4 million hectares from the previous assessment in 2006 (114). Some of this predicted loss in forest cover is the direct result of biological invasions of non-native plant pathogens or insects (49, 79, 82, 135, 146). In the United States alone, more than 450 species of insects and approximately 16 plant pathogens have been introduced since the 1800s, with severe and often irreversible impacts on forest ecosystem structure (4, 11, 37). Examples of such pathogen introductions into the United States include chestnut blight (2), white pine blister rust (69), Dutch elm disease (40), sudden oak death (116), laurel wilt (3, 112), and rapid ʻohiʻa death (58) (**Table 1**). Although there is a long history of pathogen invasions having significant adverse effects on forest ecosystems, we have had relatively limited success with their mitigation thus far.

Some of the challenges associated with controlling invasive pathogens include (*a*) the inability to identify and close pathways of introduction, (*b*) difficulty detecting introduced pathogens at low abundance in natural ecosystems, (*c*) the lack of eradication success, and (*d*) the absence of resistance in naïve plant populations (79). In terms of pathways of introduction, the live plant trade and wood packaging bear the greatest responsibility (71, 78). Although regulations have been implemented in many jurisdictions to limit the accidental import of plant pests via these pathways, considerable variation among countries still exists, resulting in porous barriers (33, 86). Given that quarantine efforts are not 100% successful, detection following an accidental introduction is the next line of defense. This approach has limited success with forest pathogens because they are difficult to detect when at low frequencies in the heterogeneous landscapes of unmanaged forests (79). Lack of early detection is linked to the breakdown of subsequent eradication efforts (12). Typically, once eradication has failed and an introduced pathogen has become established, the best available means to mitigate its impact is the breeding and deployment of disease-resistant trees (98, 130, 150). In some cases, such as chestnut blight, genetic resistance is the only option available to rescue the host tree species from extinction.

The restoration of foundation tree species to areas where they have been extirpated by introduced pathogens is a dilemma of the modern era. To meet the moment, the National Academy of Sciences, Engineering, and Mathematics has called for continued research and development of all biotechnology approaches to mitigate threats to forest health, including invasive forest pathogens, to protect ecosystem integrity (100). These tools range from traditional breeding to genome-informed breeding, genetic engineering (GE), genome editing, RNA interference (RNAi), tissue culture, biological control, and grafting (59). These approaches have been studied to varying degrees in different forest pathosystems (**Table 1**). Here, we review the ecological and economic impact of damaging introduced pathogens, focusing on examples in North America. We then synthesize the successes and challenges of past biotechnological approaches and discuss

Table 1 Disease resistance status of introduced pathogens in the forest ecosystems of the United States

Pathogen/disease	Host	Pathway of introduction	Geographic distribution in the United States	Status of disease resistance	Reference(s)
Ceratocystis lukuohia I. Barnes, T.C. Harrin, and L.M. Keith *Ceratocystis huliohia* I. Barnes, T.C. Harrin, and L.M. Keith Rapid ʻŌhiʻa death (ROD)	ʻŌhiʻa (*Metrosideros polymorpha* Gaud.)	Unknown	Hawaii	Initial screening trials complete; survivors identified in the field; a framework for a ʻŌhiʻa disease resistance program has been proposed	84
Cronartium ribicola J.C. Fisch White pine blister rust (WPBR)	All five-needle pines	Live plants	Throughout all the states along the Atlantic Coast inland to Tennessee and up through the Upper Midwest Also along the Pacific Coast inland to South Dakota and down to New Mexico	Major gene for resistance documented in four species; variation in quantitative resistance for remaining five species; restoration programs from seed of WPBR-resistant trees for several decades for eastern and western white pine and sugar pine and more recently whitebark and limber pine; candidate genes have been identified for marker-assisted and genomic selection	131
Cryphonectria parasitica (Murrill) Barr. Chestnut blight	American chestnut and chinquapin (*Castanea* spp.)	Live plants	Throughout the host native range	Transgenic blight-tolerant American chestnut and hybrids developed	115
Harringtonia lauricola (Harrington & Fraedrich) Araújo, Y. Li, and J. Hulcr Laurel wilt disease *Xyleborus glabratus* Eichhoff	Trees in the Lauraceae, especially redbay (*Persea borbonia* L. Spreng), sassafras [*Sassafras albidum* (Nutt.) Nees], and avocado (*Persea americana* Mill.)	Wood packing material (hypothesized)	Southeastern United States	Screening program initiated for redbay; results from field inoculation trials indicate variable levels of host susceptibility to pathogen	58, 108
Neonectria ditissima (Tul. & C. Tul.) Samuels & Rossman *Neonectria coccinea* var. *faginata* (Pers.) Fr. Beech bark disease Vectored by *Cryptococcus fagisuga* Lindinger	American beech (*Fagus grandifolia* Ehrh.)	Live plants	Northeastern United States	Resistance known for 40 years; major gene for resistance to scale recently identified	18, 56

(*Continued*)

Table 1 (*Continued*)

Pathogen/disease	Host	Pathway of introduction	Geographic distribution in the United States	Status of disease resistance	Reference(s)
Ophiostoma ulmi Buisman *Ophiostoma novo-ulmi* Brasier Dutch elm disease Vectored by several species of insect including *Scolytus multistriatus* Marsham and *Scolytus schevyrewi* Semenov-Tian-Shanskij	American elm (*Ulmus americana* Planch.) and other native elms in North America and Europe	Wood products	Throughout the continental United States, except the desert Southwest	Ten American elm cultivars available in North America with moderate levels of resistance; identified through cloning elm specimens exhibiting resistance in the field	40, 127
Phytophthora ramorum S. Werres, A.W.A.M. de Cock Sudden oak death	>140 species of plant, including tanoak (*Notholithocarpus densiflorus* Manos, Cannon and S.H. Oh), bay laurel (*Umbellularia californica*), and several species of oak [*Quercus* spp. (C.G.D. Nees) Nuttall]	Live plants	Central coast of California to southern Oregon in forest systems	Variation in *P. ramorum* susceptibility within tanoak and coast live oak populations documented; coast live oak resistant trees identifiable through Fourier-transform infrared spectroscopy	23, 53, 54, 132
Phytophthora lateralis Tucker & Milbrath Port Orford cedar root disease	Port Orford cedar (*Chamaecyparis lawsoniana* (A. Murray bis) Parl.), Pacific yew (*Taxus brevifolia* Nutt.)	Live plants	Pacific northwest	Evidence of both QDR and MGR resistance to the disease; seedling progeny from resistant parent trees in containerized seed orchards now being used for reforestation	129
Phytophthora cinnamomi Rands Ink disease	>1,000 plant species, including American chestnut [*Castanea dentata* (Marsh.) Borkh.]	Unknown	Widely distributed throughout the continental United States and Hawaii	TACF's *C. parasitica*–resistant American chestnut Graves BC3F3 also inherited major-effect alleles for resistance to *P. cinnamomi*; alleles for resistance to *P. cinnamomi* and *C. parasitica* are not linked	148

Abbreviations: MGR, major gene resistance; QDR, quantitative disease resistance; TACF, The American Chestnut Foundation.

the integration of genomics and biotechnology to help mitigate the effects of past and future pathogen invasions. Finally, we consider the potential risks of biotechnological interventions to forest conservation. These questions are considered in the context of the transgenic American chestnut, which is the most comprehensive example to date of how biotechnological tools have been used to address the impacts of introduced pathogens on naïve forest ecosystems.

2. ECOLOGICAL IMPACT OF INTRODUCED PATHOGENS ON FOREST ECOSYSTEMS

Large-scale mortality following the accidental introduction of forest pathogens into the United States (**Table 1**) has resulted in cascading impacts on the forest ecosystems they invade. Advances in biotechnology and genomic approaches provide opportunities to remedy the ecological harm caused by invasive forest pathogens. Below, we review the historical impacts on forest ecology caused by introduced pathogens in the United States (selected from **Table 1**) to illustrate the need for such technologies.

Chestnut blight represents one of the most geographically widespread instances of the removal of a keystone native tree species from the landscape by an introduced invasive pathogen (2). American chestnut (*Castanea dentata*), once a dominant species within its natural range throughout eastern hardwood forests of North America (15, 117), has been nearly wiped out by the introduced pathogen *Cryphonectria parasitica* (2). Prior to pathogen introduction, *C. dentata* was an abundant overstory canopy tree ranging in height from 18 to 36 m and in diameter from 1 to 1.5 m at maturity (15, 117, 144). Decay-resistant wood and rapid growth rates made it an ideal timber species (60), and the consistent yearly mast was exploited by many vertebrates and invertebrates, including humans (85). Following the initial introduction and discovery of the pathogen in New York in 1904, the disease killed nearly 90% of American chestnut trees by the middle of the twentieth century (115). Survivors are typically found as understory trees, rarely producing viable nuts, and are repeatedly infected by the pathogen (115). As such, surviving trees are relegated to a minor component of the forest, rarely surviving to maturity, and not fulfilling historical ecological roles. The loss of the resource affected the availability of food for birds, animals, and insects and likely resulted in the extinction of several species of chestnut moth (27). The extirpation of this species has also affected ecosystem processes such as nutrient cycling (32), leaf litter chemistry (134), the accumulation of woody debris (28), and forest succession. The enhanced growth rate and decay resistance of chestnut relative to other hardwood species suggest that its reintroduction may resurrect unique ecological and economic benefits. These include the consistent production of quality nuts for wildlife, valuable timber, and other ecosystem services such as carbon sequestration (28, 32, 59).

Dutch elm disease (DED) is another historical example of unprecedented changes to forest structure as a consequence of intercontinental transport and accidental introduction of forest pathogens (13, 40, 67). The American elm was a long-lived codominant species in native floodplain forests prior to the spread of DED, often reaching 175–200 years in age, 92–184 cm in diameter, and up to 44 m in height (88). In addition to being recognized as a foundational species in floodplain forests of major rivers in the northeastern and prairie regions of North America (88), the American elm (*Ulmus americana*) was regarded as the ideal street tree for its general urban hardiness, graceful vase-shaped growth, and interlacing branches that created cathedral-like street canopies (66, 67). As such, by the early to mid-1900s, most cities and towns across the United States planted the American elm in yards, parks, and along streets and roadways to the exclusion of other species (67). The loss of nearly all mature elm trees in the mid-twentieth century following the introduction of DED-causing pathogens (13) changed the structure and composition of floodplain forests as well as successional dynamics and ecosystem processes (88). In urban environments, the loss of tall, spreading elm trees resulted in increased localized summer temperature, increases in water runoff, increases in wind speed, and reduction in air-pollution filtration (67). Functionally similar species such as ash (*Fraxinus* spp.) have replaced elm in the aftermath of DED (88). However, the subsequent introduction of the emerald ash borer (*Agrilus planipennis*) has resulted in the mortality of tens of millions of ash trees (72) and, consequently, the loss of ecological redundancy and chances for recovery in these systems.

Phytophthora ramorum causes sudden oak death (SOD) in the coastal forests of California and Oregon. SOD represents one of the most devastating contemporary examples of an introduced pathogen on forest ecosystems since it was first documented in the San Francisco Bay Area in 1990 (116). Now established across approximately 500 square miles from the coastal forests of the Big Sur ecoregion of California to southern Oregon, the pathogen causes leaf and twig infections on more than 140 native and horticultural plant species (116). Model estimates suggest that *P. ramorum* has had a negative impact on 275 million trees, with mortality estimated between 21 and 43 million trees from 2012 to 2019 (22). Cumulatively, 2.2% of the total estimated number of tanoak (*Notholithocarpus densiflorus*) stems in Oregon and California have been affected (22). Unlike other forest pathosystems, in which baseline ecological data are lacking (e.g., chestnut blight, white pine blister rust), these impacts have been monitored in a permanent plot network spanning 500 miles of California's northern coast over the past 20 years (21, 91, 92). Changes in species composition following *P. ramorum*–induced tree mortality have altered decomposition rates and nutrient cycling (21). Cascading impacts of tree mortality also include increased fuel loading (140), which is modulated by time since invasion (91). For example, increased *P. ramorum*–induced mortality contributed to overstory burn severity in areas where trees died within 1–2 years. However, older infestations had greater substrate burn severity because of accumulated volumes of fuel (91). This increased substrate burn severity resulted in lower survival of tanoak trees, which rely on lignotubers for regeneration after fire (125), pointing to downstream effects of *P. ramorum* on postfire plant community composition (126). These changes alter litter inputs in soils and streams. Removal of tanoak by *P. ramorum* has been demonstrated to increase soil nitrates, affecting nutrient cycling, decomposition, and, potentially, the microbial communities of these ecosystems (21). Biotic interactions can also be affected. Tanoak trees are known to produce 200–1,000 lbs (90–450 kg) of acorns per tree per year, which is a major food source for small mammals, birds, and insects (76). Loss of acorns can have far-reaching impacts when one considers that small mammals are the primary host for ticks, and tick populations are highly correlated with outbreaks of zoonotic diseases (136). A reduction in tanoak acorns has the potential to cascade across trophic levels impacting small mammal and tick populations and, by association, zoonotic disease outbreaks. Similar effects on other community members are likely to occur, with unknown consequences, within mixed-conifer forests of the Pacific coast (76).

Phytophthora lateralis is an aggressive introduced root disease pathogen killing Port Orford cedar (*Chamaecyparis lawsoniana*) throughout its range in the Pacific Northwest of the United States. This disease was first reported in Washington in 1923 and introduced into the native range of the Port Orford cedar by 1952. Since then, it spread largely unchecked until the 1980s–1990s when management began in earnest (41). Port Orford cedar was a common riparian tree species in southwestern Oregon and northern California and the only tree found on the ultramafic serpentine soils scattered throughout this region. As the only tree species able to tolerate the high levels of heavy metals in these soils, Port Orford cedar is associated with several rare plant species (50). The wood of Port Orford cedar is the most valuable commercially harvested conifer species in the world, and its fire tolerance and decay resistance make it a valuable component of any fire-disturbed ecosystem (50). Most trees in natural stands are susceptible to *P. lateralis* and die quickly once infected. To date, management of Port Orford cedar root disease has involved excluding the pathogen from areas where it does not yet occur, minimizing spread in already infested areas, and deploying resistant trees from the Port Orford cedar root disease resistance breeding program (50).

Since its introduction from Nova Scotia in 1890 (56), the insect–pathogen complex of beech bark disease (BBD) has dramatically shifted size structure and species composition in northeastern hardwood forests (82). With mortality reaching 80–90% of mature beech stems following widespread BBD infection (9), chronically infested stands experience reduced beech dominance and vigor and dense beech thickets in post-invasion forests. These shifts negatively affect ground-layer plant biodiversity (17, 45, 111) and nutrient cycling (38, 83), including the nitrogen cycle (38, 83).

The highly destructive laurel wilt disease, which was first detected in Georgia in 2002, has spread throughout the southeastern United States, affecting tree species in the Lauraceae (58). Redbay is the most heavily impacted tree in the family, with at least 330 million mature trees killed (58). This species is one of the most common and ecologically important forest trees of the Atlantic Coastal Plain (124). Although ecological impacts have not yet been directly measured for this emergent epidemic, elevated levels of mortality on native tree species are a major cause for concern. To date, laurel wilt has spread as far west as Texas (58), and artificial inoculations have demonstrated that California bay laurel (a.k.a. Oregon myrtle) (*Umbellularia californica*) is highly susceptible to the disease-causing pathogen (39). Large-scale changes to forest structure by invasive forest pathogens can also negatively influence wildlife.

Disease impacts on wildlife are especially pronounced in the case of white pine blister rust (WPBR) because many of the host species rely exclusively on Clark's nutcracker (*Nucifraga columbiana*) for dispersal (138). All five-needle pines (subgenus *Strobus*) in North America are susceptible to *Cronartium ribicola*, which was first introduced to western North America in the early 1900s (82). The disease is primarily responsible for the population decline of high-elevation whitebark pine (*Pinus albicaulis*), which was recently listed as a threatened species under the Endangered Species Act. The effects of this disease are particularly pronounced across the Sierra Nevada and Rocky Mountains (25). In addition to the pathogen, interacting factors such as mountain pine beetle outbreaks (*Dendroctonus ponderosae*), fire, drought, and climate change also play a role (119). Maloney et al. (87) found WPBR severely reduced cone production on whitebark pine populations in the Tahoe Basin of California and Nevada, where disease incidence averaged 35%. Similarly, McKinney et al. (89) found a negative relationship between nutcracker occurrence and probability of seed dispersal and WPBR infection of whitebark pine in the northern Rockies, where disease incidence ranged from 49% to 82% and mortality ranged from 14% to 68%, respectively. The body of evidence in both California and the Rockies suggests this bird–pine mutualism may be at risk at sites severely affected by WPBR (81). Whitebark pine seeds are also a food source for another endangered species, the grizzly bear (69). In another example, BBD results in less beech nut production, which is expected to directly affect bear populations that rely on the mast before hibernation (35).

3. ECONOMIC AND CULTURAL IMPACT OF EMERGING DISEASES

As damaging forest pathogens establish and spread in new areas, the economic costs to mitigate their impacts increase exponentially (82). Often these impacts are difficult to quantify because they are indirect but are nonetheless severe. Even more difficult to quantify, but equally important to document and acknowledge, are the cultural impacts of invasive forest pathogens. We review these impacts in the United States below.

One of the most recent and comprehensive economic analyses conducted on the impacts of an invasive forest pathogen was undertaken to evaluate the cost versus the benefit of managing the SOD outbreak in southwest Oregon (47). Although this analysis found that there was no current impact of SOD on annual timber harvest export, a minimal reduction in tanoak fiber shipments, and no decline in recreation or tourism revenues, the authors nevertheless concluded that the annual USD $1.5 million expenditure for SOD management prevented greater economic hardship. Specifically, they found that funding of SOD management at a total cost of $30 million over the next 10–20 years could offset the loss of 1,200 jobs by 2028 and $580 million in wages from 2028 to 2038. Additional losses were primarily predicted to be a result of potential sanctions on southwest Oregon timber export by China, Japan, and/or Korea; loss of timber harvest by 15% and corresponding harvest tax revenue; and a decline in recreation and tourism income due to unfavorable public perception. A similar analysis was conducted in California comparing the discounted cost of oak treatment, removal, and replacement in its communities from 2010 to 2020. The analysis estimated a discounted cost of oak removal to be $7.5 million with a loss in property values to single-family homes of $135 million. The predicted costs and property value losses are substantial, but many of the damages in urban areas (e.g., potential losses from increased fire and safety risks of the dead trees and the loss of ecosystem services) are not included.

Barring the SOD example, the economic impact of introduced diseases can be much more difficult to quantify when those effects are indirect or occurred earlier in history. For the chestnut blight case, it is argued that the decline of Appalachian subsistence culture is directly linked to the loss of the American chestnut (27). Nuts from the American chestnut were heavily utilized by native people and rural residents as a dietary supplement and for trade throughout Appalachia in the early twentieth century (27). In addition, the wood made up more than 25% and 50% of all timber cut in the Southern Appalachians and Connecticut, respectively (55, 143). The bark was also a valuable source of tannic acid used in the leather industry (27).

The economic impacts of DED have been easier to quantify given widespread outbreaks and associated mortality of elm trees growing in urban environments. By the 1970s, the cost of DED in the United States was estimated to be billions of dollars per year (67). A 40-year retrospective analysis on the economic impacts of DED on nearly 107,000 American elm trees in Milwaukee, WI, found that removal and planting costs of no-management scenarios were more than double those of the management scenarios (52). DED had significant cultural impacts, given that people have cultivated elm trees (*Ulmus* spp.) for thousands of years (67). Elm wood was historically used for timber, bows, wagon wheel hubs, and water pipes, and the bark was used by native people for making rope and covering canoes (19, 127). In another example, BBD produces dense beech thickets in aftermath forests following widespread BBD infection, which causes it to interfere with regeneration of commercially valuable species such as sugar maple (107).

4. PAST BIOTECHNOLOGY APPROACHES TO MITIGATING FOREST DISEASES

Biotechnology has a broad definition: the use of biology to solve problems (59). It is often falsely equated solely with GE or genetic modification. However, it includes a suite of tools ranging from traditional breeding to genome-informed breeding, GE, genome editing, RNAi, tissue culture, biological control, and grafting (59). Past approaches have used traditional tree breeding in combination with some methods of plant propagation. Typically, genotypes of the threatened species or a closely related species with desirable traits are bred for multiple generations using controlled pollination and selection of resistant progeny (**Figure 1**). Disease screening and

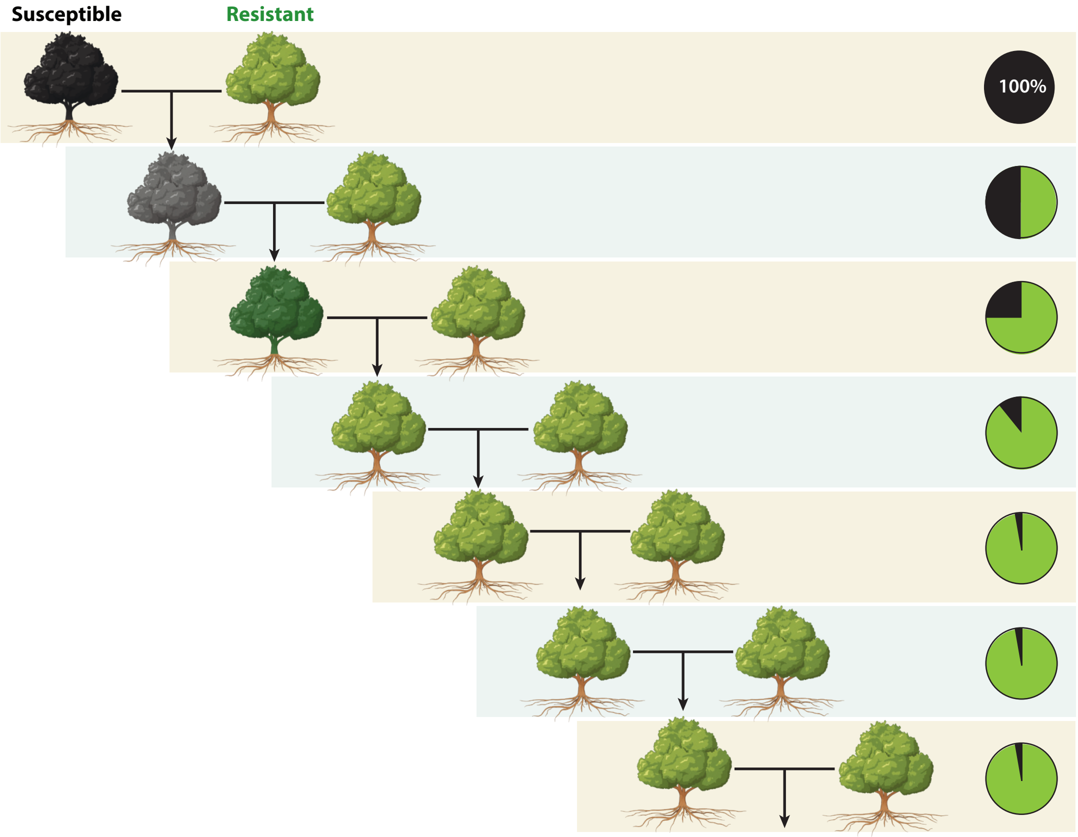

Figure 1

Disease resistance breeding exploits natural variation in plant populations to introduce disease resistance genes through classical selection or hybrid backcrossing. In a backcross breeding program, crossing begins with a resistant (e.g., Chinese chestnut) and susceptible parent (e.g., American chestnut). This is followed by several successive generations of backcrossing to the susceptible parent (American chestnut). At each generation, progeny are screened to ensure that they are resistant to chestnut blight while selecting for the desirable characteristics of the American chestnut. Figure adapted from images created with BioRender.com.

resistance breeding programs have been developed for several forest tree species, with examples highlighted below (128, 130).

Port Orford cedar has been the focus of a long-running resistance breeding program since the discovery by Hansen et al. (50) that some Port Orford cedar exhibited resistance to *P. lateralis* in greenhouse inoculations. As a result, the US Forest Service (USFS) and Bureau of Land Management (BLM), in cooperation with Oregon State University (OSU), developed a resistance breeding program to screen populations of Port Orford cedar for resistance to *P. lateralis* (41, 128, 129). To date, thousands of candidate trees, collected in wildland forests, have been screened at OSU (41). A total of 961 of the resistant Port Orford cedar are being conserved at the BLM's Tyrrell Seed Orchard, and a containerized orchard is maintained for breeding at the Dorena Genetic Resource Center. The program to develop resistance to *P. lateralis* is an excellent example whereby thousands of field selections were made initially, breeding zones created, several types of screening methodologies developed, and field trials and seed orchards established (41, 129).

Other examples of well-established classical breeding programs are the USFS efforts to restore five-needle pines to areas where their populations have been decimated by WPBR. These breeding programs have focused on two commercial species: western white pine (*Pinus monticola*) and sugar pine (*Pinus lambertiana*). Screening populations of these species for resistance revealed a wide array of resistance mechanisms (10, 69). Trees with these different resistance phenotypes have been deployed in both the United States and Canada to help mitigate the impacts of this invasive disease (69). The examples of Port Orford cedar and the five-needle pines have relied on non-coevolved resistance that arises when a trait, evolved for a different function, provides some resistance in a naïve host to a novel pathogen. This phenomenon is typically referred to as exapted resistance (44). Although effective, breeding programs are not the only biotechnology used to mitigate the impacts of invasive pathogens.

Since the introduction of the chestnut blight pathogen from Asia more than 120 years ago, many biotechnology approaches have been employed to restore American chestnut to US forests. These approaches include virus-infected hypovirulent strains of the pathogen, which fuse to healthy strains in cankered tissue and transmit the virus, slowing or stopping the disease (73). This biocontrol has been more effective in Europe than in North America, where there is a different species of chestnut (*Castanea sativa*) and the structure of the pathogen population facilitates virus transmission (93). Another approach to restoring chestnut is the planting of Japanese chestnut (*Castanea crenata*) and Chinese chestnut (*Castanea mollissima*), species that have coevolved with the pathogen and carry resistance to the disease (60, 94). However, differing ecological adaptations and the lack of natural regeneration on sites that would have supported dominant chestnut trees have limited this approach (94). In other plant species mutagenesis of seeds has been used to develop disease resistance and other agronomically desirable traits. This approach uses radiation to induce mutations that could potentially confer resistance (30). To date, thousands of irradiated American chestnut trees have been tested but none have proved to be resistant to the disease (30). Although these different approaches have met with varying levels of success, the traditional backcross breeding program, first proposed by Burnham (14), has shown the most promise (**Figure 1**).

The development of American chestnut with resistance to chestnut blight has not historically relied on exapted resistance, as described above, but rather sought to incorporate resistance from hosts that have coevolved with the pathogen in its endemic range (115). The American Chestnut Foundation (TACF) has developed backcross hybrid American chestnut for use in restoration programs (133) (**Figure 1**). Backcross trees come from conventional selective breeding of American chestnut and blight-resistant Chinese chestnut (*C. mollissima*). The hybrids were backcrossed and intercrossed over a minimum of six generations to regain most of the American chestnut phenotype while retaining the Chinese blight resistance (133). Ongoing research with modern genomic tools is continually improving the resulting trees (149). These trees are currently widely planted in the TACF research and outreach programs. This breeding program has been successful at capturing the desirable growth form of American chestnut. However, the trees are only moderately resistant to the pathogen.

These examples highlight the success of tree breeding efforts in contrast to older biotechnology solutions to mitigate the effects of biological invasions. However, there are also several drawbacks to this approach. For example, trees have extremely large genomes and are slow to reproduce, and their desirable traits are controlled by multiple genes that interact in different ways with the abiotic environment. These features result in relatively low heritability and limit genetic gain (29). In addition, a single breeding cycle can take decades to complete, and in some cases breeding for a desirable trait such as disease resistance may result in the accumulation of other less-desirable traits. For example, in the case of the backcross breeding program for American chestnut, the progeny selected for disease resistance typically have approximately 17% of the Chinese chestnut genome (149), and, as a result, never match the growth of their American chestnut parent (59). Another concern is the effect of identifying and deploying a single dominant resistance gene on a pathogen population that can reproduce sexually. This was exactly what happened in the case of WPBR. Two alleles (*Cr1* and *Cr2*) of a dominant resistance locus were identified in a population of trees near Champion Mine in western Oregon (70). Deployment of this dominant resistance gene resulted in a shift in the allele frequencies of the pathogen population, favoring genotypes that were virulent on trees with these resistance genes. The result was the eventual failure of this resistance across populations of planted western white pine and sugar pine (70). Despite these cautionary tales, all three of the breeding programs described above have been successful at identifying and deploying resistant genotypes and would only be improved by the incorporation of new biotechnology and genomic approaches.

5. NOVEL BIOTECHNOLOGY SOLUTIONS

Biotechnological advancements since the first reports of *Agrobacterium*-mediated transformation of plants in the 1980s have accelerated, becoming ever more specific and targeted (**Figure 2**). In combination with these advances, the ability to apply this technology in nonmodel systems has also expanded. Below, we describe these advances and discuss how they could be used to mitigate the impacts of pathogens.

GE is defined as inserting foreign genes or DNA fragments into cells of a target species to create a new gene sequence and can provide an alternative method for introducing disease resistance. Based on the source of the gene or DNA fragment being employed in GE, the process is referred to as transgenesis if the donor organism is not sexually compatible with the recipient

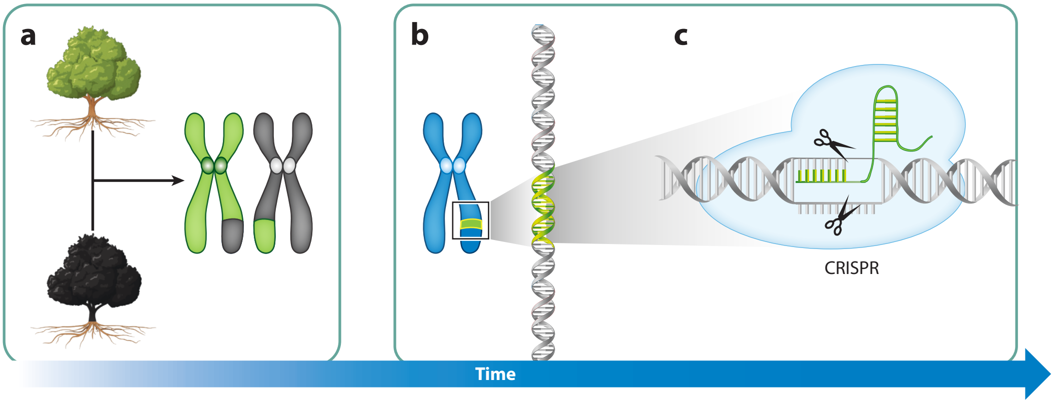

Figure 2

Progress in biotechnology specificity and accuracy. The integration of genes and traits among organisms has been occurring since the advent of agriculture. This initially began with the integration of large portions of the genome among different plants. As plant breeding and genetics were integrated, the ability to target specific genes using conventional breeding approaches also increased. A similar pattern can be observed in genetic engineering. (*a*) Initially, mutagenesis was used to cause large-scale genomic rearrangements and mutations. (*b*) Subsequently, transgenic and cisgenic approaches have been developed to insert genes from other organisms directly into the genome of a plant. Finally, genome editing is a process of genetic modification that makes specific and targeted changes to an organism's DNA. (*c*) There are several ways this can be achieved, but most of the recent advances rely on the clustered regularly interspaced short palindromic repeat (CRISPR) nuclease system. CRISPR tools are being rapidly developed, which has opened the door to using genome editing in trees and has already been developed for use in *Populus*. CRISPR technology is ideal for use in trees because it may make it possible to create homozygous biallelic DNA edits, which would eliminate the need to intercross modified trees to ensure that the edited gene was in a homozygous state. This is a significant advantage for trees, which typically have long generation times and poor tolerance for inbreeding (39). Figure adapted from images created with BioRender.com.

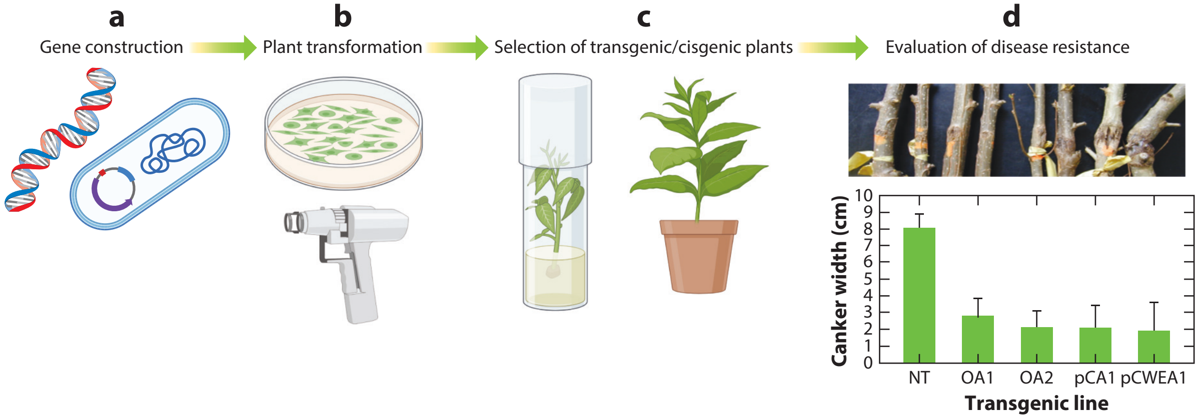

Figure 3

Inserting foreign genes or DNA fragments into cells of a target species to create a new gene sequence. This process is referred to as transgenesis if the gene or DNA fragment comes from an organism that is not sexually compatible with the recipient organism. The process is referred to as cisgenesis if the donor DNA comes from an organism that is sexually compatible with the recipient organism. Genetic engineering involves (*a*) gene construction, (*b*) plant transformation, (*c*) selection of transgenic/cisgenic plants, and (*d*) evaluation of disease resistance. The example is canker disease resistance in hybrid poplar 'Ogy' (*Populus deltoides* × *Populus nigra*) conferred by overexpression of antimicrobial peptide genes and a wheat oxalate oxidase (*OxO*) gene. The trees were inoculated with a *Sphaerulina musiva* culture plug. Data were collected after one growth season post-inoculation. Abbreviations: NT, nontransgenic; OA1, oxalic acid 1; OA2, oxalic acid 2; pCA1, a binary vector that carries the antimicrobial gene *Ac-AMP1.2* driven by a constitutive CaMV35S promoter; pCWEA1, a binary vector that carries two antimicrobial peptide genes *ESF12* and *Ac-AMP1.2* driven by a poplar wound-inducible promoter, win3.12. Data provided by Dr. William A. Powell and Dr. Charles A. Maynard. Figure adapted from images created with BioRender.com.

organism. In contrast, if the donor DNA comes from an organism that is sexually compatible with the recipient organism, the process is referred to as cisgenesis (115). *Agrobacterium*-mediated transformation and particle bombardment are the two most widely used methods for GE of plants (**Figure 3**). *Agrobacterium* species are natural genetic engineers, as they have the ability to transfer and integrate T-DNA into a plant's genome. Particle bombardment, biolistics, and/or gene gun technology are direct physical methods of gene delivery to target plant cells or tissues. This methodology uses gas pressure to insert DNA wrapped in inert metal particles, such as gold or tungsten. Generally, *Agrobacterium*-mediated transformation produces transformants carrying fewer copies of the transgene and a more predictable pattern of integration, whereas particle bombardment is a high-efficiency system that produces a large number of transgenic plants with a wide range of gene expression. The latter method seems to have some advantages with respect to federal regulations on genetically modified organisms that can stall biotech projects for years (115).

To date, no disease-resistant genetically engineered forest trees have been approved and released in forests. However, in research trials, genes have been overexpressed and tested for disease resistance (**Table 2**). Some examples include poplar, eucalyptus, American chestnut, European chestnut, American elm, and holly oak (**Table 2**). The best-documented results to date have been achieved by expressing wheat oxalate oxidase (*OxO*) in American chestnut for blight resistance (103). This gene enables degradation of toxic oxalate from the pathogen, in turn allowing the tree to coexist with the fungus in a manner similar to Asian chestnut species in the fungus' natural range (115). Currently, the *OxO*-transgenic American chestnut is undergoing review by federal regulatory agencies in the United States (1). The purpose of these trees is to rescue the surviving

Table 2 Examples of genetic engineering for improved disease resistance in forest tree species since 2001

Tree species	Pathogen/disease	Gene	Source	Reference
Castanea sativa	Root ink disease (*Phytophthora cinnamomi*)	*Ginkgobilobin-2* (*Gnk2*)	*Ginkgo biloba*	151
Quercus ilex	*P. cinnamomi*	*Ginkgobilobin-2-like* (*Gnk2*) (*Cast_Gnk2-like*)	*C. sativa*	121
Populus tomentosa	*Dothiorella gregaria*	*Aminoethoxyvinyl glycine* (*AVG*), a specific inhibitor of ethylene biosynthesis	*P. tomentosa*	80
Populus simonii × *Populus nigra*	*Alternaria alternata*	*WRKY70*	*P. simonii* × *P. nigra*	145
P. tomentosa	*Colletotrichum gloeosporioides*	*PeTGA1*, a bZIP family member	*Populus euphratica*	151
Quercus suber	*P. cinnamomi*	*CsTL1*, a thaumatin-like protein	*C. sativa*	20
Populus alba var. *pyramidalis*	*D. gregaria*, *Botrytis cinerea*	*PalbHLH1*, *PalMYB90*	*P. alba* var. *pyramidalis*	8
P. nigra × *Populus maximowiczii*	*Sphaerulina musiva*	*MsrA2*, a modified linear amphipathic α-helical host defense peptide	*Phyllomedusa bicolor*	153
Populus × *euramericana*	Canker disease (*Dothichiza populea*)	Aerobic *Vitreoscilla* hemoglobin (*VHb*), levansucrase (*SacB*), Bt endotoxin (*BtCry3A*), an anti-insect cystatin (*OC-I*), jasmonate/ethylene response factor protein (*JERF36*)	*Vitreoscilla* (*VHb*), *Bacillus subtilis* (*SacB*), *Coleoptera* (*BtCry3A*), rice (*OC-I*), wild tomato (*JERF36*)	63
P. tomentosa	*Melampsora* rust	*PtrWRKY18*, *PtrWRKY35*	*Populus trichocarpa*	63
Populus tremula × *P. alba*	Rust (*Melampsora larici-populina)*	*MYB134*	*P. nigra*	139
P. simonii × *P. nigra*	*A. alternata*	*WRKY70*	*P. simonii* × *P. nigra*	155
C. sativa	Chestnut blight (*Cryphonectria parasitica*)	*CsCh3* chitinase	*C. sativa*	24
Eucalyptus × *urophylla* × *Eucalyptus Grandis*	*Ralstonia solanacearum*, *Erwinia carotovora* ssp. *zeae* (*Sabet*), *Cylindrocladium quinqueseptatum*	N-acyl-homoserine lactone-lactonase (*aiiA*)	*B. subtilis*	110
Populus davidiana × *P. alba* var. *pyramidalis*	*Cytospora chrysosperma*, *A. alternata*	Heat shock protein (*Hsp24*)	*Trichoderma asperellum*	61
Castanea dentata	Chestnut blight (*C. parasitica*)	Oxalate oxidase (*OxO*)	Wheat	102
P. tomentosa	*D. gregaria*	*PtoWRKY60*	*P. tomentosa*	152
Picea abies	*Ceratocystis polonica*	Leucoanthocyanidin reductase (*PaLAR3*)	*P. abies*	48
Populus × *euramericana*	Canker disease (*D. populea*)	*β-1,3-glucanase*	*Populus* × *euramericana*	105
Eucalyptus urophylla	*Phytophthora capsici*	Antifungal peptide 2 (*RsAFP2*)	*Raphanus sativus*	109

(*Continued*)

Table 2 ***(Continued)***

Tree species	Pathogen/disease	Gene	Source	Reference
P. tomentosa	*A. alternata*	Chitinase (*Bbchit1*) and lipid-transfer protein (*LJAMP2*)	*Beauveria bassiana*, *Leonurus japonicus*	57
P. tomentosa	*A. alternata*, *C. gloeosporioides*	Lipid-transfer protein (*LJAMP2*)	*L. japonicus*	62
Ulmus americana	Dutch elm disease (*Ophiostoma* spp.)	Synthetic antimicrobial peptide *ESF39A*	NA	104
P. nigra × *P. maximowiczii*; *Picea mariana*	Rust for poplar (*Melampsora medusae*), root rot (*Cylindrocladium floridanum*)	Endochitinase (*ech42*)	*Trichoderma harzianum*	106
P. tremula × *P. alba*	*Xanthomonas populi*, *Hypoxylon mammatum*	Synthetic antimicrobial peptide *D4E1*	NA	90
Populus deltoides × *P. nigra*	*S. musiva*	*OxO*	Wheat	77
P. nigra × *P. maximowiczii*; *P. deltoides* × *P. nigra*	*S. musiva*	Modified chitin-binding protein gene *Ac-AMP1.2* and synthetic antimicrobial peptide gene *ESF12*	NA	77

Abbreviation: NA, not available.

remnant chestnut population by allowing introgression of the blight tolerance trait, which is generated by a single gene that can be passed on to subsequent generations through classical Mendelian inheritance (115). The same *OxO* gene has been used in the hybrid poplar line 'Ogy'. Genetically engineered trees overexpressing the *OxO* gene have reduced disease severity after one growing season following inoculation with the pathogen *Sphaerulina musiva* (**Figure 3**). These transgenes confer enhanced resistance and serve as an excellent example of how the use of genetically engineered trees has the potential to mitigate the impacts of disease.

Host-induced gene silencing (HIGS) has become a common method for engineering plant protection against pathogens and pests (75). HIGS takes advantage of widely conserved RNAi mechanisms present in most eukaryotes (122) (**Figure 4**). An early and influential example of HIGS with fungi was reported by Koch et al. (74). They demonstrated resistance to *Fusarium graminearum* in both barley and *Arabidopsis* by using HIGS to knock down the same genes targeted by azole fungicides. In another study, Wang et al. (144) demonstrated that *B. cinerea* RNAi genes (*DCL1* and *DCL2*) were also effective HIGS targets. These genes are essential for the biogenesis of small interfering RNA (siRNA) effectors that are trafficked into plants to silence host immunity genes. Observations of cross-kingdom RNAi and HIGS demonstrate that at least some fungi carry mechanisms for uptake of plant-derived double-stranded RNA (dsRNA) (16, 144, 147).

RNAi-mediated control of pests and pathogens has also been demonstrated by spraying dsRNAs directly onto plants (73, 144). This strategy, known as spray-induced gene silencing (SIGS), can be as effective as HIGS and has the potential to offer the most pathogen-specific fungicides ever developed (73, 144). With SIGS, it is thought that fungi take up dsRNA both from the leaf surface and from plant tissues after dsRNA has first been absorbed and translocated within the plant (105). Although RNAi is nearly ubiquitous in fungi (99), the ability to take up dsRNA, either from the host or the environment, may be much less common and may vary across

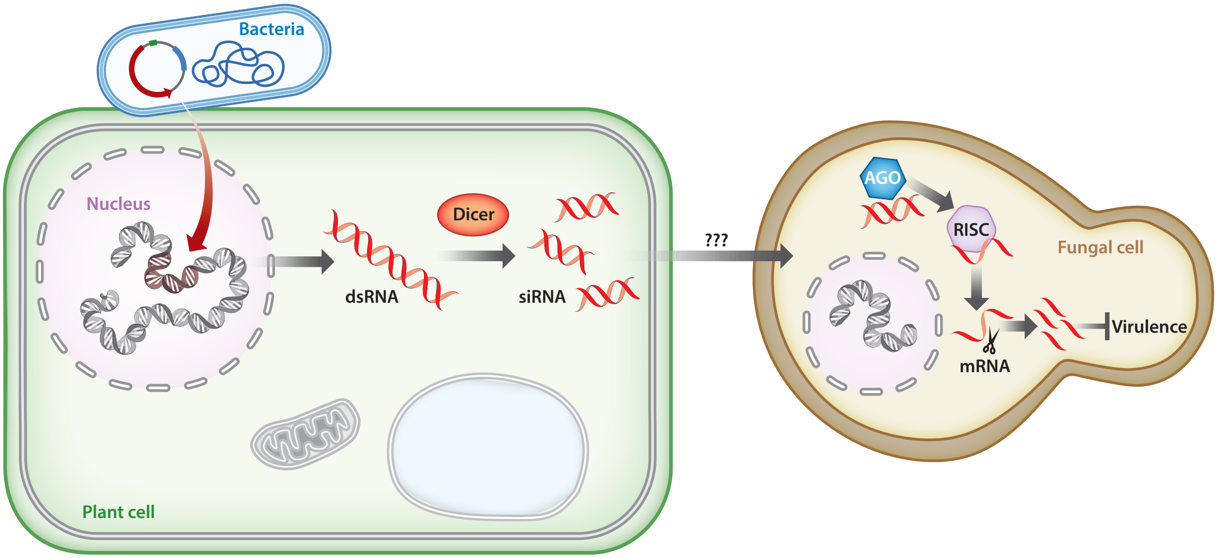

Figure 4

RNA interference is a phenomenon common to all higher organisms for defense against parasites and pathogens and the regulation of gene expression. RNAi refers to the process of post-transcriptional gene silencing of specific messenger RNA (mRNA) that is mediated by small RNA (sRNA) with complementary sequences. In host-induced gene silencing (HIGS), the sequence of the silencing RNA is carefully selected to determine which genes can be effectively silenced. The sRNAs that trigger RNAi are double-stranded RNA (dsRNA) and are processed by dicer-like proteins into small interfering RNAs (siRNAs) that are ~21–24 bp in length. siRNAs then complex with Argonaute proteins (AGO) that selectively remove one strand and use the other to form an RNA-induced silencing complex (RISC). The RISC then silences (i.e., knocks down) mRNA with complementary sequence to the siRNA by transcript cleavage or translational inhibition.

fungal species (68). It appears that dsRNA uptake mechanisms critical for HIGS and SIGS are not present in all fungi. However, the specificity of RNAi to target a specific pathogen species and not affect other associated microbes is appealing for natural ecosystems with their diverse interacting communities.

RNAi as a means of inducing resistance to tree pathogens is in the early stages of development for many host–parasite combinations. There are only two studies testing the effect of RNAi, mediated by either SIGS or HIGS, in the literature. SIGS, targeting a toxin biosynthetic pathway, in the pine tree needle disease *Dothistroma septosporum* was demonstrated to reduce symptom development on infected needles (95). In contrast, in a study testing the effect of HIGS on the fungal pathogen *S. musiva*, the cause of Septoria leaf spot and stem canker of *Populus*, it was demonstrated that the fungus was unable to take up dsRNA in vitro and that HIGS transgenic lines of *Populus*, targeting both *cyp51* and *DCL* homologs in *S. musiva*, were not more resistant to the pathogen than were wild-type trees (43). Although RNAi shows great promise, similar to agricultural systems, the effectiveness is dependent on the specific host-pathogen combination and the mechanism, either HIGS or SIGS, used to deploy the RNAi.

Similar to RNAi, the CRISPR-Cas9 genome-editing biotechnology exploits a widely conserved cellular mechanism. This mechanism evolved to protect prokaryotic cells from phages (64). CRISPR-Cas9 editing makes sequence-specific edits to genomes by inducing double-stranded breaks in DNA at specific sites (64). Two components are required for successful DNA cleavage by CRISPR-Cas9. The first is a guide RNA complex (gRNA) and the second is a Cas9 nuclease. The gRNA complex allows for genome editing to be targeted to a specific sequence in the genome. The first reports of CRISPR-Cas9-mediated genome editing in plants involved *Arabidopsis thaliana* and *Nicotiana benthamiana* (123). Since then, CRISPR-Cas9 mutagenesis has been used to edit nonmodel organisms (36, 123). The first successful CRISPR-mediated gene editing in woody plants was conducted in poplar (36). Genome-editing technology could be exploited to eliminate genes that make a plant susceptible to disease. For example, Muchero et al. (96) identified a gene that makes *Populus trichocarpa* susceptible to the fungal pathogen *S. musiva*, the cause of Septoria leaf spot and stem canker. Trees lacking this susceptibility gene are resistant to the disease. Using CRISPR-Cas9, it would be possible to edit the genome of susceptible *P. trichocarpa* trees, making their progeny resistant.

6. INTEGRATING GENOMICS AND BIOTECHNOLOGY

The sequencing of the human genome ushered in the genomics era in biology. As the ability to sequence and assemble genomes has improved and the associated cost has declined, the sequencing of populations of microbes and the host plants they colonize has become possible. This has unlocked the potential to link genes to phenotypes and accelerate disease resistance breeding. How this technology can be leveraged to combat forest health threats is described below.

The development of disease-resistant trees for restoration of extirpated species in wildland forests is fundamentally different from breeding for resistance to pathogens in agricultural ecosystems (118). Not only must resistance be heritable, but it must also be durable across a diversity of abiotic and biotic environments while being incorporated into a diversity of genetic backgrounds (59). It is not enough to develop and deploy a small number of resistant but genetically uniform individuals. To restore a species to its former distribution, it is essential to capture and incorporate the standing genetic variation within that species and exploit its adaptive potential to develop locally adapted disease-resistant populations. This objective ensures the continued survival of the species in a changing environment.

Powerful resources that can be used to meet these restoration goals are resequenced populations of host species. These populations can be used to evaluate population structure, describe the demographic history of the species, evaluate genomic diversity across genomes and populations, identify signatures of selection, and link phenotypes to genotypes (34, 42, 118). This information can be used to inform management strategies and restoration efforts. These approaches are in their infancy when it comes to mitigating forest health threats but show great promise. For example, Sandercock et al. (118) recently used a resequenced population of 384 American chestnut trees to identify management units for restoration, discover that genomic diversity decreased with latitude, and determine that populations with the greatest genetic diversity were in the southern tip of the species range. Perhaps most importantly, they provide evidence that natural selection has already enriched for putative disease resistance in the remnant American chestnut populations. These results are being used to target sampling in these remnant populations, and these individuals are then being used in breeding programs to capture their diversity and adaptive potential.

Owing to the genetic diversity and genomic complexity of populations of trees, it is extremely challenging to identify traits and link those phenotypes to their underlying genetic determinants that can be targeted in breeding programs. Genome-wide association studies (GWAS) can be applied to resequenced populations to link phenotypes to genotypes. A GWAS uses a linear mixed model to identify associations between traits and specific genes. Some examples using this approach have been used to identify loci-mediating host–parasite interactions, including resistance to WPBR in *Pinus lambertiana* (142), susceptibility to *Heterobasidion parviporum* in Norway spruce (97), and susceptibility and resistance of *Populus trichocarpa* to Septoria stem canker (96). These approaches can be leveraged further to identify loci that mediate multiple processes in the host plant. These other traits could include enzymes that mediate wood production and disease resistance (154) or disease resistance to multiple pests and pathogens (**Figure 5**). These loci can then be used as part of breeding efforts or as targets for GE, genome editing, and RNAi discussed above.

7. RISKS OF BIOTECHNOLOGY SOLUTIONS TO FOREST HEALTH AND TREE MICROBIOMES

Plants provide a complex habitat for communities of microbes within plant tissues (as endophytes) or on the surface (as epiphytes). These communities of fungi, bacteria, oomycetes, and viruses are ubiquitous throughout the plant kingdom. Direct or indirect microbe–plant–abiotic interactions collectively shape plant health (97). Although advances in biotechnology and genomics provide an unprecedented opportunity to remedy the catastrophic ecological and economic harm caused by invasive forest pathogens, their use also raises questions about whether genetic modification of trees could impact their natural microbiomes. For the first time, genetically modified trees are being planted in US forests (113). However, the effects of transgenic plants on ecosystem processes and the natural microbiomes associated with those trees have only been tested in a small number of cases (101, 120, 141). For example, Newhouse et al. (101) evaluated belowground interactions between *OxO*-transgenic American chestnuts and associated community members in two greenhouse experiments. In the first experiment, there was no effect of transgenic leaf litter on the germination or biomass of eight plant species commonly found in American chestnut–dominated forests (101). Similarly, there was no effect of the transgene on the colonization of transgenic American chestnut root tips by ectomycorrhizal fungi (101). These results are broadly consistent with previous field and greenhouse work, which indicated no difference in mycorrhizal colonization of transgenic and wild-type American chestnut trees (26, 31). In a similar study, transformation of birch with a 4-coumarate:coenzyme A ligase (*4CL*) gene involved in the biosynthesis of lignin did not affect mycorrhizal colonization or the decomposition of the litter from transgenic trees (141). In these studies, the authors proposed that the lack of an effect is likely due to two factors: the widespread distribution of OxO and 4CL enzymes in plants and the absence of specific toxic effects of these enzymes on microbes (101). Similarly, fungal community composition did not differ between transgenic and nontransgenic *Populus trichocarpa* leaves in a study involving HIGS trees expressing genes targeting the fungal pathogen *S. musiva*'s *Cyp51* and *DCL* genes (**Figure 6**). This initial body of evidence suggests that biotechnology could serve as a promising tool to mitigate disease without influencing the natural microbiomes in disease-affected trees.

In contrast, one study found that the toxin produced by transgenic hybrid poplar (*Populus tremula* × *Populus tremuloides*) possessing *Bacillus thuringiensis* (Bt) genes, which impart resistance to leaf-feeding insects, had an unexpected effect on stream invertebrate communities (6). In that study, there was an increase of 25% and 33% in insect abundance on transgenic leaf litter relative to the wild-type plants (6). Although the authors did not have an explanation for this unanticipated outcome, they proposed three possible explanations: pleiotropic effects (5–7), trophic effects on microbial assemblages (46), or prey effects (51). Regardless of the mechanism, the widespread distribution of the Bt toxin in the headwaters of streams where transgenic maize plants are planted suggests that careful selection, testing, and deployment of transgenes in natural ecosystems would be essential (137).

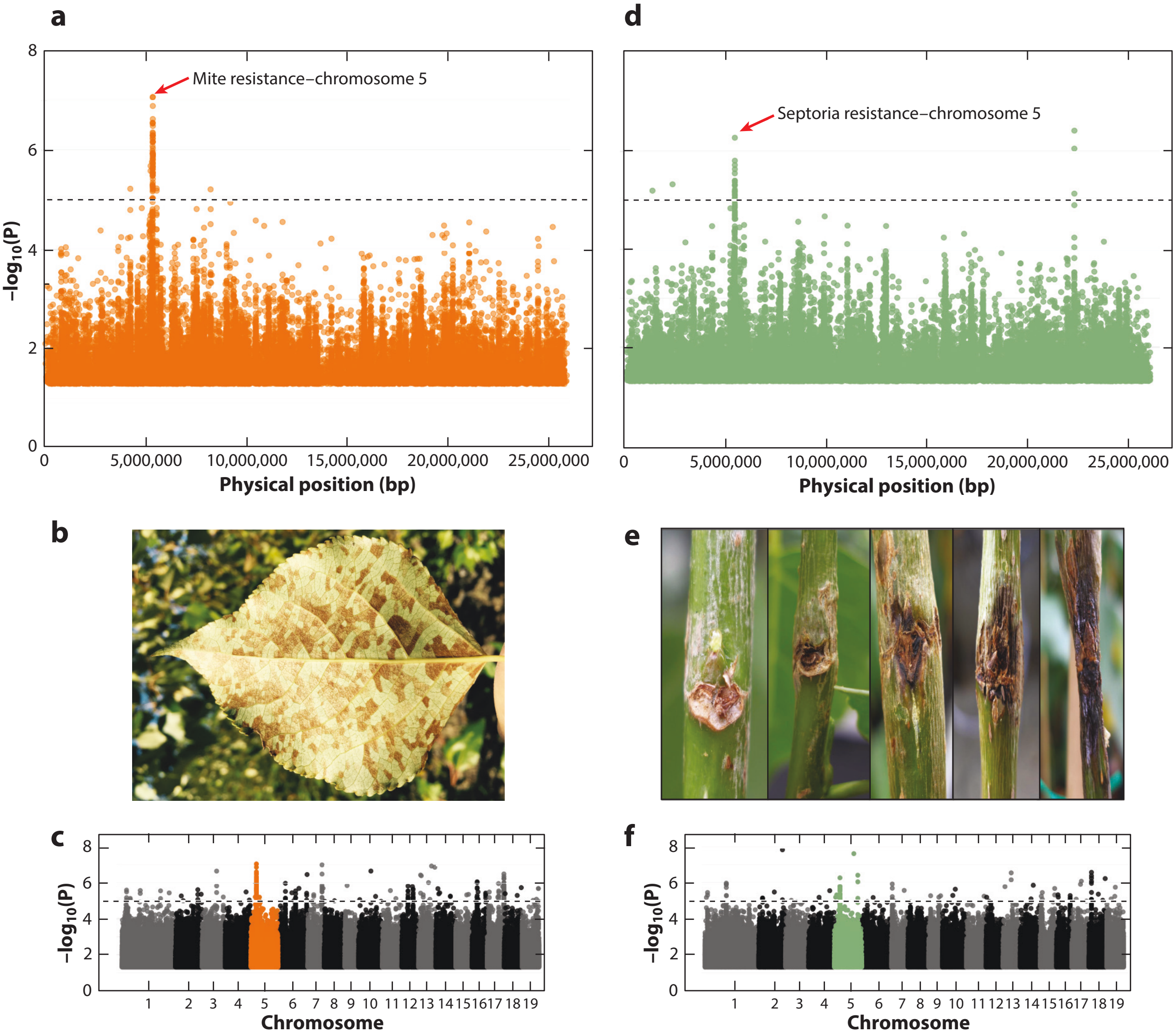

Figure 5

Disease resistance to introduced pathogens relies upon non-coevolved resistance that arises when a trait, evolved for a different function, confers some resistance called exapted resistance to the introduced pathogen (44). Combining coevolved resistance to native pathogens with exapted resistance to invasive pathogens has great potential for tree breeding. For example, resistance to the native mite pest of *Populus trichocarpa*, *Schizoempodium mesophyllincola*, is mediated by the same locus that confers resistance to the introduced pathogen *Sphaerulina musiva*. (*a*) Manhattan plot of *Populus trichocarpa* chromosome 5 depicting a significant association with an *ABC-2 type transporter* (Potri.005G073100; $P < 0.0001$) and leaf bronzing severity caused by the mite *S. mesophyllincola*. (*b*) Picture of severe leaf bronzing symptoms on a *P. trichocarpa* leaf. (*c*) *Populus trichocarpa* genome-wide Manhattan plot of significant associations. Chromosome 5 is highlighted in orange. (*d*) Manhattan plot of *Populus trichocarpa* chromosome 5 depicting significant associations of an *ABC-2 type transporter* (Potri.005G073100) ($P < 0.0001$) with Septoria stem canker severity (1–5 scale). (*e*) Disease severity scale (1–5) of *P. trichocarpa* stems inoculated with *S. musiva*. (*f*) *Populus trichocarpa* genome-wide Manhattan plot depicting all significant associations, with chromosome 5 highlighted in green. Each dot on the four Manhattan plots corresponds to a marker, its level of significance, and its physical position on the chromosome (Mb). The dashed lines represent the Bonferroni-corrected significance threshold.

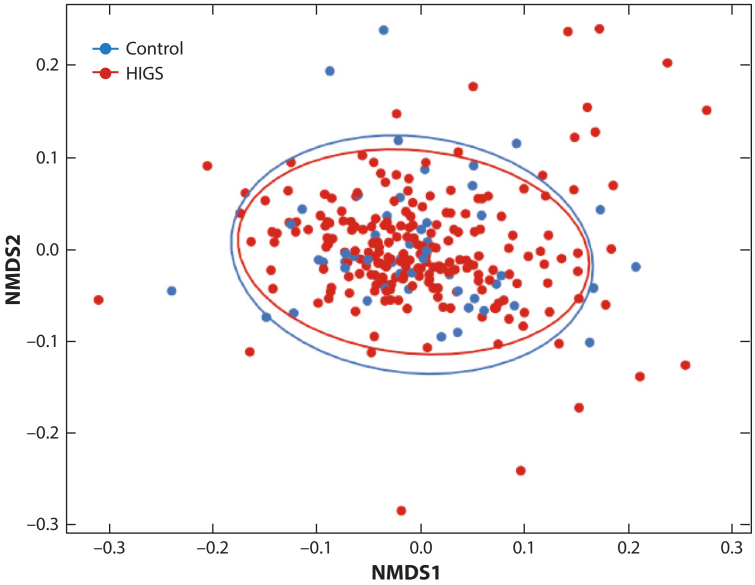

Figure 6

Biotechnology effects on tree microbiomes. One of the major concerns with genetic engineering (GE) trees is the potential impact of the transgene on the associated plant, vertebrate, invertebrate, and microbial communities. Associations with microbes are intimate and have the potential to be impacted by genetic modification. Metabarcoding of the associated microbial communities of genetically engineered and non–genetically engineered trees allows us to explore these potential impacts. These approaches are just beginning to be used to explore how GE affects a plant's microbiome. For example, the NMDS (nonmetric multidimensional scaling) ordination of trees in two-dimensional space depicts the associated fungal communities of *Populus trichocarpa* containing host-induced gene silencing (HIGS) constructs that target *Sphaerulina musiva* grown at a field site. The fungal OTUs (operational taxonomic units) ($n = 328$) were characterized using ITS2 metabarcoding. Each dot represents a fungal community from one tree after one season of growth. Red indicates samples that use HIGS to target *S. musiva* genes, and blue indicates non–*S. musiva* targeting controls. Ellipses are 95% confidence intervals around the group centroids. Fungal community compositions did not differ between *S. musiva* targeting HIGS samples and controls (PERMANOVA pseudo-F = 0.865, p = 0.488). No difference in multivariate group dispersions was found (PERMDISP, F = 0.220, p = 0.615). As expected for HIGS, a highly specific biotechnology, there is no effect of the transgene on the associated microbial community of the transgenic *P. trichocarpa*.

8. CONCLUSIONS

The restoration of foundational tree species to areas where they have been extirpated by introduced pathogens urgently needs to be addressed. The advances in biotechnology and genomics over the past decades provide us with increasingly powerful tools to meet this challenge. Given that transgenic trees are already being planted in US forests, the potential regulatory approval and release of OxO American chestnut suggest that biotechnology and genomics are likely to become integrated into how invasive pathogens are tackled in the future. The OxO transgenic American chestnut will likely serve as a roadmap and test case for the use of biotechnology to mitigate the effects of these invaders.

In conclusion, we would like to highlight what a similar biotechnology-based approach might look like for SOD. The initial steps would be the development of the genomic resources to understand and dissect the interaction between tanoak and the pathogen. A first step, which has already been completed, is the creation of a reference genome for tanoak (65). Subsequently, similar to what has been done for *P. trichocarpa* (96), an association mapping population sampled from across the range of tanoak should be developed. This resource could be used to make phenotype–genotype correlations with disease resistance, abiotic stress tolerance, and other desirable traits. This population could be utilized as part of a conventional breeding program or as targets for a variety of the biotechnology approaches discussed above. Simultaneously, a method to transform tanoak would need to be developed and tested. The risks of these genetically modified organisms to the associated community members would also need to be evaluated. Finally, a strategy for deployment would need to be developed in concert with stakeholders and regulators. None of these steps are trivial and will all require time and resources. However, the alternative would be the extirpation of another species and another ecosystem permanently altered by an introduced pathogen.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

AUTHOR CONTRIBUTIONS

Jared M. LeBoldus conceived of the review, wrote the manuscript, and helped create all the figures. Shannon C. Lynch helped conceive of the review and write the manuscript. Andrew E. Newhouse helped conceive and edit the review. Kelsey L. Søndreli, George Newcombe, Patrick I. Bennett, Posy E. Busby, and Michael Gordon helped collect, analyze, and summarize the data in sections 5, 6, and 7. Wellington Muchero and Jin-Gui Chen helped collect, analyze, and summarize the data in section 6. Haiying Liang contributed data for figures and helped write the review. All authors helped edit and revise the final version of the review.

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