Reproductive modification in forest plantations: impacts on biodiversity and society

Summary

Genetic engineering (GE) can be used to improve forest plantation productivity and tolerance of biotic and abiotic stresses. However, gene flow from GE forest plantations is a large source of ecological, social and legal controversy. The use of genetic technologies to mitigate or prevent gene flow has been discussed widely and should be technically feasible in a variety of plantation taxa. However, potential ecological effects of such modifications, and their social acceptability, are not well understood. Focusing on *Eucalyptus*, *Pinus*, *Populus* and *Pseudotsuga* – genera that represent diverse modes of pollination and seed dispersal – we conducted in-depth reviews of ecological processes associated with reproductive tissues. We also explored potential impacts of various forms of reproductive modification at stand and landscape levels, and means for mitigating impacts. We found little research on potential reactions by the public and other stakeholders to reproductive modification in forest plantations. However, there is considerable research on related areas that suggest key dimensions of concern and support. We provide detailed suggestions for research to understand the biological and social dimensions of containment technologies, and consider the role of regulatory and market restrictions that obstruct necessary ecological and genetic research.

I. Introduction

The Earth’s forests and associated biodiversity supply vast quantities of ecosystem services and products, including clean water, human and wildlife habitat, erosion control, carbon storage, medicines, and wood, chemical and energy products. Increases in the global human population (projected to grow from 7.1 to 8.3 billion by 2030) are expected to dramatically increase demand for forest products, such as timber by 129 million m³ (FAO et al., 2012). Resource extraction has traditionally come at the cost of forest loss and degradation, with a loss of c. 13 000 ha yr⁻¹ (FAO et al., 2012), which, in turn, drives biodiversity loss (Pimm et al., 2014) and declines in ecosystem services (Gamfeldt et al., 2013). Tree plantations are recognized as a partial solution (Brockerhoff et al., 2014).
et al., 2008), as they usually have higher rates of wood volume accumulation than natural forests. Approximately 35% of the world’s wood supply is generated by plantations, yet they comprise only 5% of the global forest area (FAO, 2010).

Tree breeding, increasingly accompanied by genomic methods, is ubiquitous in plantation management and is a major contributor to high rates of plantation productivity. In recent years genetic engineering (GE) – defined here as the direct and asexual alteration of DNA in living organisms – has been proposed as a new tool for tree breeding. Because GE can be used for changing native genes or transferring in novel functions, it has the potential to produce a variety of modified or novel traits. A large body of field research has shown that GE methods appear capable of delivering improvements to plantation productivity, wood processing, pest resistance, or chemical products (FAO, 2010; Haggman et al., 2013; Table 1). In some cases, the improvements can be substantial, such as elevating productivity in the field by c. 20% in poplar (Klocko et al., 2014; Lu et al., 2014; Ault et al., 2016). However, the movement of seeds and pollen from GE plantations to feral and wild populations (hereafter termed ‘gene flow’) has been highly contentious in its regulatory treatment, public acceptance and possible impacts on markets. For example, pollen ‘contamination’ of non-GE products, an important economic problem with GE crops, may be even more difficult with GE plantation trees as a result of their typically longer dispersal distances. Pollen from GE eucalypts (that might contaminate honey products) was cited as a concern related to the growing commercial use of GE eucalypts in Brazil (Petermann, 2015) – where GE reproductive modification is illegal – and was a contributing factor to vandalism of company glasshouses (Marty, 2015).

In order to mitigate the social and biological problems of gene flow, GE methods that would reduce or prevent gene flow have been developed, but their potential impacts on biodiversity and society have received limited attention. Removal of pollen and/or seeds could obviously impact pollinators, as well as nectar- and seed-consuming organisms, and thus deserves careful consideration for each potential application so that this potential fix to the problem of gene flow is used judiciously and does not needlessly reduce the benefits of GE traits, or exacerbate rather than attenuate global impacts of plantations on biological diversity.

The primary goals of this paper are to evaluate the potential ecological impacts that modifications to forest tree reproduction might have on biodiversity, contextualize these impacts by comparing them to effects of other forms of forest management on reproductive activity, and examine how reproductive modification technology is expected to affect public, interest group and government responses. We focus primarily on the potential biodiversity and social impacts of GE reproductive modification; the impacts of other types of GE plantation modifications and the potential consequences of gene flow from GE plantations (when allowed or encouraged) have been discussed elsewhere, including in a recent compendium volume on GE forest biotechnologies (Vettori et al., 2016).

### Table 1 Examples of field trials of genetically engineered (GE) trees that have showed efficacy or promise in delivery of target traits

<table>
<thead>
<tr>
<th>Tree species</th>
<th>Trait</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Chestnut Castanea dentata</td>
<td>Fungal blight resistance</td>
<td>Maynard et al. (2009); Zhang et al. (2013)</td>
</tr>
<tr>
<td>American Elm Ulmus americana</td>
<td>Dutch elm disease resistance</td>
<td>Newhouse et al. (2007); Sherif et al. (2016)</td>
</tr>
<tr>
<td>Silver Birch Betula pendula</td>
<td>Fungal rust resistance</td>
<td>Pasonen et al. (2004)</td>
</tr>
<tr>
<td>Poplar Populus tremula × alba</td>
<td>Biomass allocation</td>
<td>Lu et al. (2015)</td>
</tr>
<tr>
<td>P. tremula × alba</td>
<td>Tree size</td>
<td>Elias et al. (2012)</td>
</tr>
<tr>
<td>P. tremula × alba</td>
<td>Improved pulpalibility</td>
<td>Pilate et al. (2002); Coleman et al. (2012); Mansfield et al. (2012); Pilate et al. (2002)</td>
</tr>
<tr>
<td>P. tremula × alba</td>
<td>Decreased lignin</td>
<td>Franke et al. (2000); Costa et al. (2013)</td>
</tr>
<tr>
<td>P. × canescens</td>
<td>Specialty chemical production</td>
<td>Costa et al. (2013)</td>
</tr>
<tr>
<td>P. tremula × alba</td>
<td>Reduced isoprene emissions</td>
<td>Behnke et al. (2012)</td>
</tr>
<tr>
<td>P. trichocarpa × deltoides, P. tremula × alba, P. tremula × tremuloides, P. trichocarpa × nigra</td>
<td>Nitrogen assimilation</td>
<td>Jing et al. (2004); Meilan et al. (2002); Ault et al. (2016)</td>
</tr>
<tr>
<td>P. nigra, P. deltoides × nigra, P. trichocarpa × deltoides</td>
<td>Herbicide tolerance</td>
<td>Meilan et al. (2002); Ault et al. (2016)</td>
</tr>
<tr>
<td>P. davidiana × bolleana</td>
<td>Insect resistance</td>
<td>Hu et al. (2001); Klocko et al. (2014)</td>
</tr>
<tr>
<td>P. alba</td>
<td>Flowering control</td>
<td>Klocko et al. (2016b)</td>
</tr>
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</table>

### II. Impacts of reproductive modification

We begin by outlining methods for GE of reproduction in forest trees. Then, to help interpret the significance of GE modifications to reproduction, we discuss the extent that forest management itself impacts tree reproduction. Next, we discuss the extent that various organisms utilize reproductive resources in our target genera. We conclude by considering the social factors that could influence acceptance or rejection of GE reproductive modification. Our methods for literature searches related to biodiversity and social dimensions are provided in Supporting Information Notes S1. These searches were structured to gather information related to a number of explicit hypotheses (Notes S2).

#### 1. GE reproductive modification

There are a variety of potential and demonstrated options for GE-based reproduction modification in trees (reviews in Brunner et al., 2007; Vining et al., 2012). In addition to the goal of containment, reproductive modification also has been sought as a means to improve vegetative growth by increased allocation of photosynthetic resources (Strauss et al., 1995), reduce the production of
allergenic pollen and decrease nuisance reproductive tissues in urban trees. Most published studies of GE have focused on male-sterility because pollen is the main source of long-distance spread and seed propagation would allow for continued sexual breeding. One method for obtaining male-sterility, genetic ablation (i.e. cell elimination), was first developed and commercialized in agriculture, and is effective and reliable in field trials of Populus, Eucalyptus and Pinus (Zhang et al., 2012; Elorriaga et al., 2014; Fig. 1). However, because many intensively grown forest trees, most notably poplars and eucalypts, have seeds that can travel large distances by wind, water or animals, research also has focused on achieving complete bisexual (male and female) sterility. Both poplar and eucalypt plantations are widely established via vegetative cuttings, such that loss of seed production will still allow for tree propagation and establishment using current methods. Published results for achieving female or bisexual sterility have emphasized suppression of key flowering genes by RNA interference (Klocko et al., 2016b; Fig. 2), a method that can be highly stable in field-grown trees (Li et al., 2008). Direct editing of floral genes by use of CRISPR-Cas nucleases or similar approaches should be even more stable as entire genes can be deleted (Elorriaga et al., 2015), but to our knowledge, gene-edited trees have yet to be tested in the field. Other methods that have been proposed for trees, but not yet demonstrated in the field, include the use of developmentally inducible recombinases to excise (i.e. to selectively remove from the genome) GE-modified genes during the formation of gametes. This approach would allow normal flowering yet little or no release of transgenes. Genes that suppress the age-related onset of reproduction also are potential targets for breeding or GE, as a wide array of floral suppressor genes has been identified in basic plant research (Pajoro et al., 2014).

A common concern is that containment will be incomplete or unstable, or that asexual forms of dispersal will still allow for gene flow (Brunner et al., 2007). In poplar, which has been widely studied, field research and simulations show that even incomplete or unstable male and female sterility would still provide high levels of genetic containment in various scenarios, including when linked to a strong fitness-promoting gene such as one giving resistance to an important herbivore of poplar (DiFazio, 2002; DiFazio et al., 2012). Vegetative spread was also found to be a weak means of dispersal compared to pollen and seed. It is possible to modify organisms to prevent or slow vegetative spread, such as by preventing rooting except in the case of gene induction. However, to our knowledge, such methods have never been shown to be effective and vegetative spread can often be readily controlled by forest management rather than genetic methods (e.g. by scouting for and removing root and branch sprouts within and near to plantations). It also appears feasible to engineer post-flowering forms of containment by targeting genes required for pollen tube growth or fertilization, or genes that take part in embryogenesis. Many such genes have been characterized in model plants (Wang et al., 2007; De Smet et al., 2010), which might enable the production of floral and fruit organs with essentially normal nutritive value for biodiversity, but without the possibility of propagule growth and development. However, to our knowledge, such methods – although widely discussed for crops and for which there are a number of patents for reversible forms – have not been developed or field-tested in crops or trees (Lombardo, 2014). Given the wide scope for continued genetic innovation as knowledge of genes, development and methods for genetic modification grow, we can expect to see many refinements and new forms of genetic containment technologies in the future.

2. Impacts of forest management on reproductive activity

Intensive forest management practices often strongly influence reproductive activity. Perhaps the most extreme examples are short rotation bioenergy plantations that are managed on a one- to few-year coppice system (Santangelo et al., 2016). Trees such as poplars are densely planted and literally cut-off near ground level during annual or semi-annual harvests, allowed to re-sprout from the roots, and then cut and re-sprouted for multiple harvest cycles.

Fig. 1 Barnase expression leads to male sterility in field-grown trees. Male catkins of Populus tremula × tremuloides clone INRA 353-53 showing pollen release in Petri dishes from (a) control but not from (b) barnase trees (S. H. Strauss, unpublished; scientific background in Elorriaga et al., 2014). Eucalyptus occidentalis flowers of (c) control and (d) barnase (note shriveled anthers that are not releasing pollen). (e) Pinus rigida × taeda male cone clusters of GUS control (left) and barnase (right) in pollen isolation bags. Control flowers shed large quantities of pollen grains, whereas pollen grains were absent from barnase flowers. Panels (c–e) reprinted with permission from Zhang et al. (2012).
Given this early harvest, trees are kept in a largely juvenile phase of fast growth with little or no flowering. However, even in longer rotation forest plantation systems, trees normally do not become reproductively active for several years, and the high planting density common to most systems (to suppress weeds, promote height growth and stimulate branch pruning), further delay the onset of reproduction and reduce the intensity of flowering and fruit production.

Thinning, or low natural stocking, has been associated with increased reproductive activity in a number of studies. For example, Verkaik & Espelta (2006) reported that Aleppo pine (\textit{Pinus halepensis} Mill.) showed two- to six-fold increases in the number of new cones produced per tree in thinned stands compared to unthinned stands. Thinning also led to a four-fold increase in the percentage of newly reproducing trees. Benefits of thinning can last for 5 yr or more, as in \textit{Pinus taeda} (Kozlowski et al., 2012). This common trend (i.e. negative correlation between reproduction and stand density) was supported in 15 of 18 studies on conifer species (Table 2). Exceptions to this trend were observed in both wild stands (Moya-Laraño et al., 2007; Ruano et al., 2015) and seed orchards (Copes & Bordelon, 1994), perhaps because densities already were quite low, or as a result of other environmental factors known to affect flowering intensity (e.g. fertilization and irrigation in seed orchards).

Intensive genetic selection for rapid growth can further reduce the onset or intensity of reproduction, especially where propagation systems do not bias propagules toward those that flower early and most intensively (e.g. young seed orchards). In maritime pine \((\text{Pinus pinaster})\), Santos-del-Blanco \textit{et al.} (2015) reported that a single cycle of selection for timber yield decreased the proportion of reproductive trees (51% in selected group vs 66% in control group), delayed the age and size at first reproduction, lowered the reproductive-to-vegetative allocation ratio, and affected the proportion of protandrous vs hermaphroditic individuals.

3. Stand-level biodiversity impacts

\textbf{Modifying tree reproduction and changes in resources} In order to interpret the potential impacts of flower modification on biodiversity, we need to be able to interpret how the impairment of plant reproduction can influence resources, which may then influence communities and ecosystem processes in a diversity of ways (Fig. 3). Impairing flower reproduction may alter the availability of pollen, nectar, fruits and seeds. Understanding both how tree reproductive resources are used by animals, and the nature and timing of changes in reproductive activity, are important for interpreting potential effects and may vary widely among taxa (Fig. 4). For example, alterations of male vs female flowering, changes in the time of year that flowering occurs and changes in the year of onset during a tree rotation will have differential impacts on dependent organisms. As discussed above, genetic methods can bring about different floral modifications, including a complete absence of flowers, pollen-less flowers, and (at least in theory) ecologically normal flowers, fruits and seeds whose pollen and seed are unable to give rise to viable progeny, or lack transgenes.
Table 2 Impact of stand density on flowering and seed production.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stand type</th>
<th>Measurement</th>
<th>Density</th>
<th>Density/reproduction association</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aleppo pine (Pinus halepensis Mill.)</td>
<td>Wild</td>
<td>Cone production per tree</td>
<td>800–130000 trees ha(^{-1})</td>
<td>Negative</td>
<td>Alfaro-Sánchez et al. (2015)</td>
</tr>
<tr>
<td>Aleppo pine (Pinus halepensis Mill.)</td>
<td>Wild</td>
<td>Cone production, seed production</td>
<td>n/a</td>
<td>Negative</td>
<td>Reviewed by Ayari &amp; Khouja (2014), which discussed studies done by Goubitz et al. (2004), Espelta &amp; Verkaik (2007), Moya et al. (2008, 2009) and Ayari et al. (2012)</td>
</tr>
<tr>
<td>Aleppo pine (Pinus halepensis Mill.)</td>
<td>Wild</td>
<td>Cone production per ha</td>
<td>7089–61 145 trees ha(^{-1})</td>
<td>Positive</td>
<td>Moya-Laraño et al. (2007)</td>
</tr>
<tr>
<td>Loblolly pine (Pinus taeda)</td>
<td>Wild</td>
<td>Cone production per tree, Seed production per tree</td>
<td>n/a</td>
<td>Negative</td>
<td>Reviewed by Barnett &amp; Haugen (1995), which discussed studies done by Wenger (1954) and Grano (1970)</td>
</tr>
<tr>
<td>Loblolly pine (Pinus taeda)</td>
<td>Wild</td>
<td>Flower bud production per tree, cone production per tree</td>
<td>n/a</td>
<td>Negative</td>
<td>Kozlowski et al. (2012)</td>
</tr>
<tr>
<td>Loblolly pine (Pinus taeda)</td>
<td>Plantation</td>
<td>Cone production per tree, number of seeds per cone, seed viability</td>
<td>Average stocking ranged from 50 to 770</td>
<td>Negative (cone production per tree vs stand density); None (number of seeds per cone and seed viability vs stand density)</td>
<td>Florence &amp; McWilliam (1954)</td>
</tr>
<tr>
<td>Slash pine (Pinus elliottii)</td>
<td>Wild</td>
<td>Cone production per tree, seed production per tree</td>
<td>n/a</td>
<td>Negative</td>
<td>Reviewed by Barnett &amp; Haugen (1995), which discussed studies done by Cooper &amp; Perry (1956), and Halls &amp; Hawley (1954)</td>
</tr>
<tr>
<td>Slash Pine (Pinus elliottii)</td>
<td>Plantation</td>
<td>Cone production per tree, cone production per acre, number of seeds per cone, seed viability</td>
<td>Average stocking ranged from 60 to 320</td>
<td>Negative (cone production per tree vs stand density); Positive/negative (cone production per acre vs stand density; increased with closer spacing when stocking &lt;100–120 trees per acre, then decreased when tree spacing became smaller); None (number of seeds per cone and seed viability vs stand density)</td>
<td>Florence &amp; McWilliam (1954)</td>
</tr>
</tbody>
</table>
Table 2 (Continued)

<table>
<thead>
<tr>
<th>Species</th>
<th>Stand type</th>
<th>Measurement</th>
<th>Density</th>
<th>Density/reproduction association</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortleaf pine (Pinus echinata Mill.)</td>
<td>Wild</td>
<td>Percentage of sound seeds per tree</td>
<td>Released: basal area $14 \text{ m}^2 \text{ ha}^{-1}$; Unreleased: basal area $28 \text{ m}^2 \text{ ha}^{-1}$</td>
<td>Negative</td>
<td>Grayson et al. (2002)</td>
</tr>
<tr>
<td>Shortleaf pine (Pinus echinata Mill.)</td>
<td>Wild</td>
<td>Cone production per tree, seed production per tree</td>
<td>n/a</td>
<td>Negative</td>
<td>Reviewed by Barnett &amp; Haugen (1995), which discussed studies done by Phares &amp; Rogers (1962) and Grano (1970)</td>
</tr>
<tr>
<td>Longleaf pine (Pinus palustris)</td>
<td>Wild</td>
<td>Cone production per tree</td>
<td>n/a</td>
<td>Negative</td>
<td>Reviewed by Barnett &amp; Haugen (1995), which discussed studies done by Allen (1953) and Croker (1952)</td>
</tr>
<tr>
<td>Maritime pine (Pinus pinaster Aiton)</td>
<td>Wild</td>
<td>Seed density, increase in seed density</td>
<td>45–188 trees $\text{ ha}^{-1}$</td>
<td>Positive (seed density vs stand density); Negative (increase in seed density vs stand density)</td>
<td>Ruano et al. (2015)</td>
</tr>
<tr>
<td>Red pine (Pinus resinosa)</td>
<td>Wild</td>
<td>Cone production per tree</td>
<td>n/a</td>
<td>Negative</td>
<td>Wisconsin Department of Natural Resources (2010)</td>
</tr>
<tr>
<td>Western white pine (Pinus monticola)</td>
<td>Seed production area</td>
<td>Strobilus production per tree, seed production per tree, sound seed per cone</td>
<td>n/a</td>
<td>Negative (strobilus and seed production per tree vs stand density); None (number of sound seeds per cone vs stand density)</td>
<td>Barnes (1969)</td>
</tr>
<tr>
<td>Hoop pine (Araucaria cunninghamii)</td>
<td>Plantation</td>
<td>Cone production per tree, number of seeds per cone</td>
<td>Average stocking ranged from 100 to 600</td>
<td>Negative</td>
<td>Florence &amp; McWilliam (1954)</td>
</tr>
<tr>
<td>Spanish fir (Abies pinsapo)</td>
<td>Wild</td>
<td>Cone production per tree, number of seeds per cone, seed viability</td>
<td>n/a</td>
<td>Negative (cone production vs stand density); Positive (number of seeds per cone and seed viability vs stand density)</td>
<td>Arista &amp; Talavera (1995)</td>
</tr>
<tr>
<td>Douglas fir (Pseudotsuga menziesii)</td>
<td>Seed orchard</td>
<td>Cone production per tree, seed production per tree</td>
<td>Tree spacing 12 ft vs 24 ft within rows spaced 24 ft apart</td>
<td>None</td>
<td>Copes &amp; Bordelon (1994)</td>
</tr>
</tbody>
</table>

n/a, not available.
The relative impacts of floral modification by GE will depend on the plantation system in place and on natural environmental variation. Even without GE floral modification, resources from flowering can be extremely rare, either due to short-rotation harvests occurring largely before reproductive onset (e.g. bioenergy plantations; Munsell & Fox, 2010) or when flowering occurs late in a rotation or at a low level due to high stand density, as discussed above. In these situations, resources from reproduction are limited or absent in existing plantations, such that flower modification may have negligible consequences for altered resource distribution. In many plantations, resources from reproduction such as pollen, nectar and short-lived seeds may only be available during brief times of year, such that those resources are only available to certain members of the community and for a restricted period of time (Table 3). Across plantation tree species, the type of reproductive output, its quantity (e.g. seedfall or biomass of pollen) and its nutritive value, will vary. Natural environmental variation can also exert strong effects on reproductive resources, both due to variation in weather (e.g. moisture stress; Yuccer et al., 2003) and climatic cycles. Many tree species fail to reproduce for many years then flower heavily in others (masting), with flowering associated with complex climate and weather variation (e.g. Sakai et al., 2006). Many conifers, including our focal genera Pinus and Pseudotsuga, have well-known masting cycles (Koenig & Knops, 1998). In
addition to this temporal variability, monoculture plantations also represent a spatial aggregation of floral resources. In agricultural contexts, monocultures of mass-flowering crops can impact pollinators both positively and negatively (Westphal et al., 2006), implying that floral modification in mass-flowering tree plantations may also have impacts on pollinator populations, pollination services and honey production.

Resource consumers and biodiversity changes Although searches including terms for GE reproductive modification yielded >400 results, we found no empirical studies of impacts of GE reproductive modification on biodiversity, thus all of the inferences below are based on studies of reproduction–biodiversity relationships in conventional plantations or wild stands.

Classifying animals into functional groups, rather than considering them taxonomically, facilitates understanding of the potential effects of resources changes on biodiversity. We analyze functional groups in terms of the morphological component(s) of the plant reproductive tissues that are fed upon. We begin with nectar (nectarivory) and pollen (palynivory) feeders, which we functionally lumped together with pollinators because they typically consume nectar and/or pollen rewards. We then consider florivory, which we define as consumption of whole or partial flowers before seed coat formation (McCall & Irwin, 2006). When pollination is successful, a range of organisms feed on seed (spermivory) and fruit (frugivory), both pre- and post-dispersal. These categories are helpful in understanding the animal taxa involved, but species often feed on multiple stages of plant reproductive tissues. In discussing the taxa potentially impacted, we highlight economically and culturally important species, as well as those that are legally protected. Organism–tree genus associations from the literature are summarized in functional groups in Table 4.

Nectarivory and palynivory Many consumers of nectar and pollen also are pollinators, typically defined as animal pollen vectors (i.e. they move pollen between different flowers). Pollinators represent a substantial component of animals that feed on plant reproductive tissues, however, not all nectarivores and palynivores are pollinators. There are cases where flower visitors consume pollen and/or nectar without transferring pollen between flowers. One example of this is nectar robbery where a flower visitor circumvents flower opening to remove nectar without contacting anthers or stigmas (Irwin et al., 2010). Pollination as an interaction likely evolved from palynivory by beetles (Labandeira & Eble, 2000) although palynivory without at least some incidental pollen transfer may be rare given that most palynivores will visit multiple flowers and likely carry some pollen on their bodies. The exception is in monoecious or dioecious plants where palynivores may be able to focus exclusively on male flowers or plants. Three of our focal genera – Pinus, Populus and Pseudotsuga – are wind-pollinated and thus do not produce floral nectar (Fig. 4; Table 3). These taxa likely have a community of palynivores, although we could find no studies characterizing the pollen-feeding assemblages of these taxa.

The primary tree genus that is animal-pollinated and also the focus of transgenic developments is Eucalyptus, which has a diverse and substantial community of flower visitors that vary depending on geographic location and species. This list includes marsupials, birds and a diverse assemblage of insects (Table 4). In plantations without active pollination management, seed production is often low and pollen-limited (reviewed in Moncur et al., 1995), which may result in reduced seed consumer abundance and/or richness compared with other land uses. Most Eucalyptus species provide copious and sugar-rich nectar resources (Moncur et al., 1995), and beekeepers in many parts of the world will bring honey bee colonies to plantations during flowering to promote honey production.

Fig. 4 Reproductive structures of Pinus, Populus, Pseudotsuga and Eucalyptus. Pistilate cones and catkins of (a) Pinus taeda, (b) Pseudotsuga menziesii and (c) Populus alba. Staminate cones of (d) P. taeda, (e) P. menziesii and staminate catkins of (f) P. deltoides. (g) Eucalyptus globulus produces fruit and perfect flowers. Photograph in (c) by author Amy Klocko. Source for other images: http://bobklips.com/treeflowersmenu.html.
Table 3  Key reproductive characteristics and phenology of *Eucalyptus, Pinus, Populus* and *Pseudotsuga* that influence resources for animals

<table>
<thead>
<tr>
<th>Tree genera</th>
<th>Species</th>
<th>Pollination</th>
<th>Seed dispersal</th>
<th>Age of first reproduction (yr)</th>
<th>Flowering period</th>
<th>Seed dispersal period</th>
<th>Years between seed production</th>
<th>Serotiny</th>
<th>Vegetative reproduction</th>
<th>Flowers</th>
<th>Nectar</th>
<th>Fruit</th>
<th>Seed weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eucalyptus</em></td>
<td><em>globulus</em></td>
<td>Zoophilous</td>
<td>Anemochory</td>
<td>3–4</td>
<td>Year-round</td>
<td>Year-round</td>
<td>Year-round</td>
<td>None</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Capsule 0.002</td>
</tr>
<tr>
<td></td>
<td><em>grandis</em></td>
<td>Zoophilous</td>
<td>Anemochory</td>
<td>2–3</td>
<td>April–May in Australia, August–November in USA</td>
<td>January–April</td>
<td>Year-round</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Capsule 0.002</td>
</tr>
<tr>
<td></td>
<td><em>reignans</em></td>
<td>Zoophilous</td>
<td>Anemochory</td>
<td>20</td>
<td>January–May</td>
<td>January–April</td>
<td>1</td>
<td>Present</td>
<td>Present, weak</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Capsule 0.006–0.013</td>
</tr>
<tr>
<td><em>Pinus</em></td>
<td><em>taeda</em></td>
<td>Anemophilous</td>
<td>Ambochory</td>
<td>5–10</td>
<td>Feb–April</td>
<td>Oct–Dec</td>
<td>3–13</td>
<td>Present</td>
<td>None</td>
<td>None</td>
<td>Rare/none</td>
<td>Cone</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td><em>radiata</em></td>
<td>Anemophilous</td>
<td>Ambochory</td>
<td>5–10</td>
<td>Jan–Feb</td>
<td>Midwinter</td>
<td>.</td>
<td>Present</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Cone</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td><em>sylvestris</em></td>
<td>Anemophilous</td>
<td>Ambochory</td>
<td>5–15</td>
<td>May–June</td>
<td>Dec–March</td>
<td>4–6</td>
<td>Present</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Cone</td>
<td>0.029–0.033</td>
</tr>
<tr>
<td></td>
<td><em>elliottii</em></td>
<td>Anemophilous</td>
<td>Ambochory</td>
<td>7–12</td>
<td>Jan–April</td>
<td>Sept–Nov</td>
<td>1–3</td>
<td>Present</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Cone</td>
<td>0.0001–0.0002</td>
</tr>
<tr>
<td><em>Populus</em></td>
<td><em>tremuloides</em></td>
<td>Anemophilous</td>
<td>Anemochory</td>
<td>2–3</td>
<td>March–June</td>
<td>March–June</td>
<td>1</td>
<td>None</td>
<td>Present</td>
<td>None</td>
<td>Extrafloral</td>
<td>Catkin</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td><em>deltoides</em></td>
<td>Anemophilous</td>
<td>Anemochory</td>
<td>5–10</td>
<td>Feb–April</td>
<td>May–July</td>
<td>2</td>
<td>None</td>
<td>Present, uncommon</td>
<td>None</td>
<td>Extrafloral</td>
<td>Catkin</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td><em>trichocarpa</em></td>
<td>Anemophilous</td>
<td>Anemochory</td>
<td>10</td>
<td>March–June</td>
<td>May–July</td>
<td>3</td>
<td>None</td>
<td>Present</td>
<td>None</td>
<td>Extrafloral</td>
<td>Catkin</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td><em>menziesii</em></td>
<td>Anemophilous</td>
<td>Anemochory</td>
<td>12–15</td>
<td>March–May</td>
<td>October–May</td>
<td>7</td>
<td>None</td>
<td>Present</td>
<td>None</td>
<td>Extrafloral</td>
<td>Cone</td>
<td>0.011–0.014</td>
</tr>
</tbody>
</table>

Table 4 Count of organisms associated with reproductive structures/products of *Eucalyptus*, *Pinus*, *Populus*, *Pseudotsuga*

<table>
<thead>
<tr>
<th>Taxonomic group</th>
<th>Organism association</th>
<th><em>Eucalyptus</em></th>
<th><em>Pinus</em></th>
<th><em>Populus</em></th>
<th><em>Pseudotsuga</em></th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birds</td>
<td>Catkin feeder</td>
<td>.</td>
<td>.</td>
<td>. 1</td>
<td>.</td>
<td>Jakubas et al. (1993), Lindroth &amp; St Clair (2013)</td>
</tr>
<tr>
<td></td>
<td>Nectivore, Palynivore</td>
<td>2</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>MacNally &amp; Horrocks (2000), Hingston et al. (2004a)</td>
</tr>
<tr>
<td>Fungi</td>
<td>Palynivore</td>
<td>. 2 .</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>Hutchinson &amp; Barron (1997)</td>
</tr>
<tr>
<td>Invertebrates</td>
<td>Catkin feeder</td>
<td>.</td>
<td>.</td>
<td>. 1</td>
<td>.</td>
<td>Mound (1998)</td>
</tr>
<tr>
<td></td>
<td>Hyphaevore</td>
<td>1</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>Hingston et al. (2004a), Griffin et al. (2009), Menz et al. (2015)</td>
</tr>
<tr>
<td>Mammals</td>
<td>Catkin feeder</td>
<td>.</td>
<td>.</td>
<td>. 1</td>
<td>.</td>
<td>Willson et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Frugivore</td>
<td>. 1 .</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>Mikich &amp; Liebsch (2014)</td>
</tr>
<tr>
<td></td>
<td>Nectivore</td>
<td>1</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>McCartney et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Nectivore, Palynivore</td>
<td>1</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>Dobson et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Palynivore</td>
<td>1</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>van Tets &amp; Hubert (1999)</td>
</tr>
<tr>
<td>Microbiota</td>
<td>Palynivore</td>
<td>. 2 .</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>Masclaux et al. (2011)</td>
</tr>
</tbody>
</table>

Numbers represent a summation of records noting species and/or genera associated with reproductive structures/products. In some cases, multiple records came from the same reference. Reference order corresponds to organism count order in each row. References separated by commas indicate multiple references corresponding to one count. References corresponding to distinct combinations of feeding mode and tree species are separated by semicolons.
Florivory Although florivory includes consumption of, or damage to, pollen and ovules (or immature seeds) in addition to other floral structures (e.g. bracts, petals, pistils, stamens), here we exclude strict pollen/ovule consumption from florivory as already discussed. Florivory has been relatively understudied (McCall & Irwin, 2006) and there is little information on how florivory impacts the tree genera that are the focus of this review. One exception is the Douglas Squirrel (Tamiasciurus douglasii), which is known to consume entire mature pollen cones of Douglas-fir (Pseudotsuga menziesii; Maser et al., 1981). Another exception is Populus catkins, as several bird species use them for food. Cedar Waxwings (Bombycilla cedrorum) consume male catkins of Eastern Cottonwood (Populus deltoides). In experimental feeding trials, Witmer (2001) showed that captive waxwings lost body mass on a diet of Viburnum opulus fruits unless their diet was supplemented by P. deltoides catkins, whose pollen was shown to be an important source of supplemental protein. Similarly, quaking aspen (Populus tremuloides) flower buds and catkins comprise a significant portion (66–100%) of the winter and spring diet of the ruffed grouse (Bonasa umbellus; Jakubas et al., 1993), although its high amounts of secondary metabolites also reduce its nutritional value. Various bird species may also use Populus catkins indirectly as a source of invertebrate food. For example, long-snouted weevil (Dorytomus spp.) larvae feed on pollen grains within staminate catkins and may provide a supplemental food source for birds during energy-intensive migration (Leatherman, 2011).

Spermivory Seeds and plant ovules represent a rich resource utilized by various animals. We treat this separately from frugivory, or fruit consumption, which often also involves seed consumption, and which is covered below. Seed and ovule feeding (hereafter referred to as ‘seed’ for simplicity) can take place in various floral developmental stages and ecological contexts. Seeds can be consumed pre- or post-dispersal (Howe & Smallwood, 1982), by insect larvae that develop exclusively within a single seed and by vertebrates that consume many seeds over the course of a single day. In general, the mode of seed dispersal is of ecological importance for spermivory, given that wind- (or gravity-)dispersed seeds (including Pseudotsuga, Populus and Eucalyptus) tend to be of low nutritive value relative to most animal-dispersed seeds (including those of Pinus; e.g. Gautier-Hion et al., 1985).

Two of our focal tree genera – Populus and Eucalyptus – are not considered animal dispersed and have relatively non-nutritious seeds that do not serve as a substantial resource for animal communities. For example, Eucalyptus globulus is considered gravity-dispersed and most seeds are estimated to fall within 20 m of the parent plant (Skolmen & Ledig, 1990). A study of this species in Spain, where it is an exotic, found no evidence of any seed predation or animal-mediated seed movement (Calviño-Cancela & Rubido-Bará, 2013). Similarly, Populus has small wind-dispersed fruits with single seeds that are unlikely to be an important food source for vertebrates (Zasada et al., 1992).

However, the dichotomy between animal dispersal and wind/ gravity dispersal is not perfect, and of one our focal genera, Pseudotsuga, is a counterexample. Its seeds are winged and thought to be dispersed primarily by wind and gravity (e.g. Fowells, 1965), but are food items for: a number of mammals including mice, voles, shrews and chipmunks (Gashwiler, 1970); birds including the Winter Wren (Troglodytes hiemalis), Pine Siskin (Carduelis pinus), Song Sparrow (Melospiza melodia), Golden-Crowned Sparrow (Zonotrichia atricapilla), White-Crowned Sparrow (Zonotrichia leucophrys), Dark-Eyed Junco (Junco hyemalis) and Purple Finch (Haemorhous purpureus) (Arno & Hammerly, 1977); and a range of insect seed predators (Strothmann & Roy, 1984). Relatively specialized feeders on Pseudotsuga seeds include the aforementioned Douglas Squirrel (Arno & Hammerly, 1977) and potential subspecies of red crossbill, including the Douglas-fir Red Crossbill (Loxia curvirostra neogaea; Parchman et al., 2006).

Of our focal tree genera, spermivory is likely the most important in Pinus. Pine seeds are considered to be wind- or gravity-dispersed in c. 85 of the 110 species in the genus, with the remaining 25 or so considered to be primarily animal dispersed, including the well-known Clark’s Nutracker (Nucifraga columbiana)–Whitebark Pine (Pinus albicaulis) symbiosis (Tomback & Linhart, 1990). A rich community of animals feed on pine seeds (‘pine nuts’ to human consumers), including from species that are considered wind-dispersed (e.g. Vander Wall, 2008). This includes several species of conservation concern including the Tenerife Blue Chaffinch (Fringilla teydea), a species endemic to Spain that primarily eats Pinus sylvestris seeds (Garica-del-Rey et al., 2011). Similarly, the Maroon-fronted Parrot (Rhynchopsitta terrisi) and Thick-billed Parrot (Rhynchopsitta pachyrhyncha) are spermivores native to the southwest USA and Mexico that feed primarily on seeds from pines such as Pinus ponderosa, P. menziesii, Pinus engelmannii and Pinus arizonica (Snyder et al., 1994).

Frugivory Frugivory, or consumption of fruit, technically only occurs in angiosperms because gymnosperms do not produce fruit (they are named for their naked (‘gymno’) seed (‘sperm’)). Thus, seed consumption in gymnosperms, including Pinus and Pseudotsuga, is considered spermivory. Of our target species, frugivory in the angiosperms – Eucalyptus and Populus – is not substantial, as discussed above under spermivory.

Specialization on resources The degree that species specialize on reproductive resources will influence the extent that altering reproduction will impact populations and communities. In
general, it is frequently acknowledged that highly specialist pollinators (Waser et al., 1996; Bascompte et al., 2003) and frugivores (Ottewell et al., 2014) are rare. This is particularly the case in temperate systems where interannual and seasonal variation in flower and fruit availability makes specialization an evolutionarily risky strategy (Waser et al., 1996). For those species that do specialize on these resources, flower modification may have negative impacts. We expect that specialization will be less likely to occur when plantations use non-native tree species, but this will depend partially on evolutionary similarity between tree species and native species in the area, and the length of cultivation in the new geography. In addition, specialists may be uncommon in plantation forests (Bremer & Farley, 2010), such that flower modification may have few impacts because these species may already be absent.

4. Landscape-level impacts

Management practices often have effects beyond those observed at local scales; composition and configuration of habitats can have a considerable influence on biodiversity at regional and global scales (Turner, 1989; Haddad & Tewksbury, 2005). Such effects become particularly pronounced as landscapes become more homogeneous (i.e. beta-diversity is reduced; Van der Plas et al., 2016). Given uncertainty about the degree that GE-induced changes to tree reproduction may influence habitat quality across taxa, here we consider five potential scenarios for landscape-scale effects: (1) creation of low-quality or nonhabitat, (2) plantations as permeable matrix, (3) the emergence of ecological traps, (4) changes in nutrient cycling; and (5) impacts on local and regional climate.

GE plantations as low-quality or nonhabitat  Removal of reproductive structures is most likely to render habitats of reduced quality, or nonhabitat, for pollinators and seedeaters. Much has been written on the effects of habitat loss and fragmentation on native biodiversity over the past 20 yr. Ultimately, it is the spatial extent of habitat loss due to GE plantations across the landscape that will determine compatibility with biodiversity (Fahrig, 1998; Hanski, 1998). If GE plantations result in only minor habitat loss at the landscape scale (e.g. <5–20%), we consider population-level effects to be highly unlikely (e.g. Andrén, 1994; Fahrig, 2003). At these levels of habitat loss, population declines should be marginal and remaining habitat would tend to be highly connected, which facilitates inter-patch dispersal. However, both theoretical (Fahrig, 1998) and empirical work (Andrén, 1994; Betts et al., 2007) have indicated that thresholds exist where habitat decline results in disproportionate population declines. Mechanisms for such thresholds are many and include reduced dispersal capacity once landscapes become highly fragmented by nonhabitat (Hanski, 1998), Allee effects (i.e. positive density dependence; Fletcher, 2006; Schmidt et al., 2015), and increased risks of predation and parasitism (Robinson et al., 1995). The amount of GE plantation development that can take place at the landscape level before it compromises gamma diversity (i.e. the regional species pool; Ricklefs, 1987), ecosystem functioning and population viability of native species depends on the dispersal capacity of organisms to recolonize managed stands. In turn, this will be a function of the spatial configuration of plantations and the degree to which they impede animal movement (Root & Betts, 2016).

GE plantations as permeable matrix  Although reproductively modified GE plantations may not serve as breeding habitat for pollen- and seed-dependent animals, for some species the cover provided by tree canopy may facilitate movement among patches (Brockerhoff et al., 2008). In such cases, a matrix dominated by plantations could be preferable to other types of habitat loss that would occur through urbanization or agricultural development. We predict that mature forest-associated species are more likely to benefit from this positive ‘matrix’ effect. Conversely, for species that preferentially breed and disperse though early seral habitats (e.g. butterflies and many species of pollinators; Haddad & Tewksbury, 2005), closed-canopy GE plantations are more likely to constitute a barrier, thereby potentially reducing population viability.

GE plantations as ecological traps  Organisms regularly depend on environmental cues to make habitat selection decisions. However, in anthropogenically altered environments, previously reliable cues may become maladaptive. In such cases, organisms can become ‘trapped’ by their behavioral responses, resulting in reduced survival or reproduction (Schlaepfer et al., 2002). Given the novelty of GE reproductive modification, and the lack of studies about how organisms that feed on reproductive structures use them as cues, little is known about the degree that such plantations could constitute ecological traps. If native species select habitat based on structural cues associated with the forest rather than direct cues such as the pollen or seeds (as food), the potential exists for GE plantations to act as traps. In such cases, even small amounts of GE plantations could potentially have negative impacts on populations because individuals could be drawn from otherwise appropriate habitat toward suboptimal locations with reduced fitness. Simulated population models (Schumaker et al., 2004) could assist in understanding the degree of ‘trap’ effect required in relation to other demographic parameters before substantial risks to populations are encountered. However, the biological knowledge on which such models must be built is presently lacking, in no small part due to the rarity of all types of GE plantations, reproduction-modified or otherwise (c. 0.00033% of plantation area: less than c. 500 ha, mostly poplars in China (Walter et al., 2010; Häggman et al., 2013; Vettori et al., 2016)), in relation to a global plantation estate of c. 150–200 million ha (of total forest area of c. 4 billion ha; R. Sedjo, pers. comm., May 18, 2016).

Role of pollen in nutrient cycling  Enriched with vegetation growth-limiting elements such as nitrogen, tree pollen may provide a significant supplement of macronutrients to forest ecosystems early in the growing season. For example, Perez-Moreno & Read (2001) found that a species of ectomycorrhizal fungus (Paxillus involutus) was able to consume nitrogen and phosphorus from the extensive pollen deposited by a member of our focal genera (P. sylvestris) and return a significant proportion of these nutrients to its host tree Betula pendula. They demonstrated experimentally that Betula individuals grown with pollen and mychorrizal
symbionts contained significantly more nitrogen and phosphorus than those grown without pollen, resulting in increased seedling growth. However, compared to litterfall, overall nutrient inputs into forest ecosystems via pollen appear to be small. For example, Cho et al. (2003) found pollen deposition in temperate pine forests in Korea to be c. 0.3% that of total litterfall by weight, with nitrogen inputs corresponding to 1% and 6% of total nitrogen and phosphorus, respectively. Pine pollen may nonetheless be a significant source of nutrients during the critical early growing period. In single-species plantations of Pinus and other wind-pollinated species, however, such inputs would likely be the same or less than the loss of nutrients from pollen release.

Tree pollen may provide an important source of nutrients for freshwater ecosystems. Graham et al. (2006), for example, found that Pinus pollen enhanced productivity in a small (0.27 km$^2$) boreal lake system by increasing phosphorus and carbon availability. From May to June, an estimated 7 kg km$^{-2}$ of pollen (0.5% P by weight) was deposited onto the lake, providing a potential subsidy of 10 kg of phosphorus. The increased levels of phosphorus (and correlated increases of carbon) were associated with significant increases in diatom, phytoplankton and zooplankton abundance around the time of pollen deposition.

Role of pollen in regional climates Changes in the abundance and types of tree pollen may significantly influence local and regional climate. Bioaerosols, including pollen grains present in large quantities, can alter cloud properties and influence atmospheric radiative forcing by scattering and absorbing solar and planetary radiation (Bonan, 2008; Després et al., 2012). Pollen also has been shown to be an efficient source of cloud condensation nuclei (e.g. Pope, 2010; Steiner et al., 2015), but its relative importance to cloud formation is unclear given that it is often at low density and of short duration compared to other natural and anthropogenic sources of such nuclei. As with most other reproductive modifications, the climatic impacts of modified pollen production would be highly scale- and context-dependent.

5. Social dimensions

Support and opposition for GE In addition to biological considerations, societal responses toward GE and related technologies can impact their adoption and regulation (e.g. Davison et al., 1997; Frewer et al., 2003, 2004; Costa-Font et al., 2008; Gupta et al., 2011; Lucht, 2015). Although specific studies on societal responses to reproductive modification in trees are scarce, research associated with more general use of GE in trees provides insight into whether people might reject or accept reproductive modification.

Concepts including attitudes, risk, knowledge and trust are related to acceptance of GE (Fig. 5). Attitudes involve evaluating an entity such as GE with some degree of favor or disfavor (Fishbein & Ajzen, 2010). Compared to agriculture, attitudes toward GE in trees and forests are less understood and there are only a few studies of attitudes about reproductive modification in forest trees (Strauss et al., 2009). In a sample of students in multiple countries (mostly European), Kazana et al. (2015) found generally positive attitudes toward using GE of trees in plantations, but not in wild natural forests. In a sample of the public in the USA, Needham et al. (2015) found that attitudes were less favorable toward using GE in wild forests compared to other forestry techniques (e.g. breeding, planting, thinning), but GE was more favorable for addressing tree diseases (e.g. chestnut blight) compared to other forest impacts (e.g. climate change, increasing growth for timber; Fig. 6). GE also was more acceptable in native species compared to adding genes from exotics or distantly related species (Needham et al., 2015). Attitudes toward GE also vary between experts (e.g. forest scientists, forestry agencies) and the public, with experts often having more favorable attitudes (Małyska et al., 2014; Porth & El-Kassaby, 2017 New Phytologist Trust.)
Greatest potential for conflict, and small bubbles (PCI$_2$ = 0) represent the greatest amount of consensus and least potential for conflict. Large bubbles (PCI$_2$ = 1) represent the least amount of consensus and greatest potential for conflict, and small bubbles (PCI$_2$ = 0) represent the greatest amount of consensus and least potential for conflict.

Fig. 6 Public and expert attitudes to forest health interventions in the USA. Survey results of public ($n = 278$) and expert ($n = 195$; scientists, agencies, companies and nongovernmental organizations involved in forest issues) attitudes toward using different interventions for addressing various forest health impacts (Needham et al., 2015). Attitudes represent a composite index measured with four items on semantic differential scales (bad – good, foolish – wise, harmful – beneficial, disagree – agree; Cronbach’s alpha reliability = 0.89–0.97). The middle of each bubble represents the index mean on the vertical scale. The size of the bubble represents the Potential for Conflict Index (PCI$_2$; Vaske et al., 2010), where large bubbles (PCI$_2$ = 1) represent the least amount of consensus and greatest potential for conflict, and small bubbles (PCI$_2$ = 0) represent the greatest amount of consensus and least potential for conflict.

2014). Needham et al. (2015) found that, compared to the public, experts (scientists, agencies, companies and nongovernmental organizations involved in forest issues) had more positive attitudes toward GE in forestry (Fig. 6).

Risks and benefits of GE Most research on public responses to GE has focused on risk perceptions, which are subjective evaluations of threats posed by hazards (Slovic, 2000). In general, people express concern and skepticism toward new and unknown hazards that are not well-understood (Finucane & Holup, 2005; Rastogi Verma, 2013; Blancke et al., 2015). Given its novelty, reproductive modification in trees is likely to be associated with risk factors such as newness, harm, dread, unnaturalness and unfamiliarity (Slovic, 2000; Sjöberg, 2004). Research examining public responses to tree breeding with related species (e.g. American chestnut with non-native Asian chestnut) was considered riskier than transgenic approaches (i.e. inserting genes from unrelated species such as from bread wheat to the American chestnut), suggesting that perceptions of unnaturalness or familiarity may elicit different concerns because Asian chestnut species, although more closely related to the American chestnut than wheat, are not as familiar to the public as wheat (i.e. source of bread). Conversely, Kronenberger et al. (2014) compared perceptions toward various GE applications and found more concern when interspecies boundaries were crossed (‘transgenics’).

Sjöberg (2004) identified interfering with nature and severity of consequences as two of the most important dimensions of risk perceptions related to GE. Reproductive modification of forest trees might be viewed similarly, especially given the potential biodiversity impacts outlined earlier. Reproductive modification may be viewed as particularly unnatural because it represents a fundamental change to the natural process of procreation. This viewpoint has been emphasized by monikers such as ‘terminator genes’ in activism campaigns against reproductive modification technologies and their potential impacts on ownership of these technologies (Lombardo, 2014).

There have been only a few studies examining perceived risks associated with GE in trees. Tsouergiannis et al. (2016) compared consumer behaviors associated with GE paper products, woody biomass energy products and other wood products, and found that public safety issues and environmental impacts were among the largest concerns. Kazana et al. (2015, 2016) examined perceptions toward transgenic forest plantations and found that potential for gene escape causing contamination of wild forests (i.e. gene flow between transgenic plantation trees and wild trees) was the greatest perceived risk. Other concerns included susceptibility to disease from lignin content modification and impacts from higher herbicide inputs (i.e. from use of herbicide resistance traits). Lorentz & Minogue (2015) examined risks of eucalypt plantations and also found invasion potential and associated negative impacts on natural forests as primary concerns. These findings are consistent with Friedman & Foster (1997) who surveyed forest agency employees and found that loss of adaptation and genetic diversity, and changes in ecosystem components, were the largest perceived risks toward artificial regeneration in trees. Likewise, Needham et al. (2015) found that members of the public were most likely to agree that changing existing genes in trees and adding transgenes (from wheat or other distant species) will change the genetics of wild or native trees and cause long-term negative impacts that are currently unknown. Gene-flow risks, however, could be mitigated or avoided by the genetic containment mechanisms that are the focus of this paper. Thus, information and education about this new capability might affect risk perceptions.
Although risk perceptions dominate the literature, acceptance of reproductive modification also may be related to the extent that people view these applications as beneficial (e.g., high growth rates, control invasive species). Kazana et al. (2015, 2016) investigated perceptions of students (mostly European) toward GE trees in plantations and found that respondents perceived several benefits, including reductions in pesticide inputs, restoration of contaminated soils and higher tree productivity. These benefits, however, may differ based on the scale of production (e.g., plantation owners vs smaller community forests).

Knowledge and trust associated with GE Given the complexity of reproductive modification, most people are likely to be uninformed and lack knowledge about this topic. Yet, knowledge and awareness are often related to positive attitudes and acceptance of GE (James, 2004; Brossard & Nisbet, 2007; Huffman et al., 2007; Connor & Siegrist, 2010). In the context of transgenic trees, Kazana et al. (2015) examined self-assessed knowledge and found that over 60% of students (mostly European) said they understood the meaning of transgenic trees and were willing to purchase GE products. However, fewer than half were aware of whether these trees could be deployed in plantation forestry (e.g., grown commercially, sold on the market). The investigators also believed there to be ‘a serious perceived lack of knowledge about potential benefits and risks of the cultivation of transgenic forest trees’ (Kazana et al., 2015).

Given the technical nature of reproductive modification and likelihood that public knowledge about this topic is low, trust in knowledgeable experts (e.g., forest scientists, agencies) is an important consideration for understanding perceptions and acceptance. Trust is the willingness to rely on those responsible for making decisions or taking actions affecting public wellbeing (Siegrist, 2000). The public relies on trusted sources (e.g., scientists, agencies) to assess complex and unknown issues, and greater trust generally is associated with lower perceived risks, higher perceived benefits, more positive attitudes and higher acceptance (Siegrist & Cvetkovich, 2000). Sources such as agencies, companies and interest groups that are trusted by some people also can use information campaigns to influence perceptions and persuade people to support or oppose GE in various contexts (e.g., on private vs public land, plantations vs wild forests).

III. Synthesis

Although reproductive modification using GE may have benefits for productivity or containment of exotic species on its own, we consider that its primary use will be to facilitate the use of GE for other traits that would increase productivity, reduce losses to stressors, or improve wood-processing efficiency. Thus, an important question is whether benefits of GE are sufficient to offset the added costs to breeding of having sterile or partly sterile trees, and if economic costs of technology, regulatory approval and market limitations are sufficient to justify use of GE in general and reproductive modification in particular. Technology costs are considerable barriers, as many species and genotypes are difficult and costly to transform under present technology, further emphasizing the need for large benefits to justify GE. However, most published studies of the benefits of GE have only been of small-scale field studies of a few genotypes under highly controlled conditions, and the strong market-based preclusions to field studies make it unlikely there will be significant new, large-scale public research in the near future (Strauss et al., 2015). GE, therefore, is likely to remain very limited in scale, potentially enabling considerable research on the ecological impacts of reproductive modification and other GE traits before their possible large-scale use.

1. Land sharing vs sparing

Conservation biologists are increasingly debating the benefits of land sparing (high-yielding agriculture on small land area) or land sharing (low-yielding wildlife-friendly agriculture covering more land) (Kremen, 2015; Phalan et al., 2016). GE tree plantations have the potential to provide considerable production benefits, and such innovations are akin to developments that have occurred since the beginning of the agricultural revolution, which enabled high levels of food production on small land areas. However, those innovations resulted in large changes to the genetic composition of wild relatives of crops in many areas, as well as large reductions of general biological and genetic diversity within what became farms; similar potential changes via GE appear to be less acceptable today, presumably due to it being viewed as a more artificial process or the lack of knowledge on how prior innovations arose and have benefitted humanity. Central to the debate about whether GE plantations will provide indirect conservation benefits is whether growing more trees per unit area will ultimately result in more land set aside for conservation. Interestingly, there appears to be only a weak tendency for increased crop productivity to translate into higher rates of nature conservation in agricultural systems (Ewers et al., 2009). Improved crop-growing efficiencies likely decrease per unit production costs, which could boost demand and provide further incentives for agricultural expansion into previously undeveloped areas. Thus, intensification needs to be coupled with ‘strong governance’ or zoning that requires parallel land conservation efforts (Kremen, 2015). Zoning approaches are not new to forest management. Seymour et al. (1999) proposed ‘triad’ methods where a forested land base is divided into three zones: intensively managed, extensively managed and protected areas. Several modeling exercises have explored the ‘optimal’ relative proportions of these zones (e.g., Macean et al., 2009; Ward & Erdle, 2015), but clearly such decisions will depend greatly on the forest type and biodiversity goals under consideration, as well as the relevant political, social and economic contexts.

With respect to GE plantations, optimal amounts of different management types will depend greatly on the amount and configuration of habitat required by native species, as well as whether they are capable of moving through and/or breeding in GE plantations with reproductive modification. In addition, the large structural and species changes within the intensively modified plantation systems where GE is likely to be most used are, in general, already complete or underway, and have already had large impacts on reproductive resources—with or without GE added. To the extent that such systems are already part of intentional or
unintentional ‘triad’ systems to support biodiversity will to a large extent determine whether the added impacts of reproductive or other GE modifications are judged to have significant additional biological impacts. Such ‘zoning’ systems are commonly in place in many areas due to government controls on land uses or market-based controls such as from forest certification systems.

2. How should the Precautionary Principle be applied?
At first glance, following the ‘Precautionary Principle’ to biodiversity conservation would seem to necessarily require limited implementation of GE plantations, including those with modified reproduction. However, if this technology results in reduced land area required for wood production, or increased economic efficiency with spin-off benefits for society and environment – as has been argued forest plantations have done in many places, and that field trial experience with GE trees suggest could likely be delivered (discussed above) – application of the Precautionary Principle is by no means straightforward. As demand for wood products continues to expand, the risk of not using GE may come in form of increased rates of harvest in primary forest, conversion of native forests into non-GE plantations, and increased impacts to wild and planted forests from climate change-associated abiotic and biotic stressors. Thus failing to develop GE options could be viewed as being in opposition to the Precautionary Principle (Strauss, 2015). It is therefore critical that future policy decisions on GE be informed by the full suite of trade-offs between traditional forestry practices and implementation of GE plantations. Understanding the trade-offs, of course, requires continued research, both of GE benefits and biodiversity impacts. Unfortunately, both types of research have become difficult as a result of regulation and market obstacles (primarily forest certification systems) to field plantings (Strauss et al., 2015). This preclusion is unfortunate as certification systems, such as PEFC, point to a lack of adequate research on GE trees as a reason for their exclusion: ‘... as the scientific evidence of potential benefits and dangers of genetically modified organisms (GMOS) and its impact on biodiversity remains insufficient and the society has not completed its debate, the PEFC General Assembly has determined that GMO cannot be considered as part of PEFC certified material’ (PEFC International, 2016). A critique of the Forest Stewardship Council’s GE policy, which was the first to ban all planted GE trees with no exemption for research, was first published in 2001 (Strauss et al., 2001).

IV. Research priorities
There are a number of research avenues to improve understanding of the possible paths and impacts of reproductive modification in plantation trees. A few of the highest priority needs are briefly discussed below. A full list is provided in Notes S3.

1. GE reproductive modification
Both the science and technology underlying GE forestry is in its infancy; basic genetic and genomic science on which GE is based continue to expand rapidly. Perhaps the most promising path to new and more effective methods for reproductive modification is through direct gene editing, such as by the CRISPR-Cas system, which can be used for mutating genes required for male and/or female reproduction, and can be reversed if needed for further breeding. Such mutations should be highly if not perfectly stable, and after initial research to establish efficacy, can be identified and selected (based on rapid DNA analysis) years before natural flowering occurs (i.e. as soon as GE shoots are produced in the laboratory). Trees with desired changes can undergo accelerated flowering studies of their effects in the laboratory (e.g. using FT gene overexpression technology; Klocko et al., 2014) followed by field studies of their impacts on reproductive and vegetative development. These studies should test for possible off-target effects and instability, improve delivery and removal methods, and monitor for pleiotrophic effects on tree growth and development. Among the target genes should be those that are active late in reproduction, such as those required only during pollen germination and embryo development. In theory, knock-outs of the latter types may produce flowers, and even fruits and seeds, that can serve as normal resources for organisms that feed on them.

2. Biodiversity
Our literature searches have revealed an absence of studies directly addressing the effects of reproductive modification on plantation biodiversity. Thus, the most urgent need is for field sampling and experimental perturbations to analyze the extent of use, and dependence of plantation biota, on reproductive structures. This knowledge is needed for informing the design of hypothesis-driven stand and landscape-level experiments, and models of what landscape impacts might be and how they could be mitigated. It is also needed to help interpret the biological and social significance, and trade-offs, associated with reproductive modification compared to the many other human impacts on biological diversity. By understanding effects on biodiversity, this information also will provide insight into other ecosystem services that may be altered. Given there are so few GE plantations established around the world – and no species commercialized with reproductive modification – there appears to be ample time for surveys and experiments to inform management should social and political conditions become conducive to wider use of GE plantations.

3. Social dimensions
If reproductive modification is to be used for managing gene flow from GE and exotic plantations, it will be essential to understand what drives public attitudes and perceptions of benefits and risks. It also will be important to understand how reproductive modification is perceived by different stakeholders (e.g. public, experts, NGOs), as gene flow is itself a major concern voiced by opponents of GE in forestry. An important element of acceptance is likely to be familiarity. Given scientific uncertainty around reproductive modification, fear of unknown potential impacts will likely continue until science can shed light on these impacts and effectively communicate them in the context of other impacts on
biodiversity (e.g. from agriculture, urbanization, plantations) and similar types of common technological interventions (e.g. neutering pets, birth control, seedless fruits, GE foods). Investigating benefits and costs of GE in forest plantations will also require ethical and political considerations such as how much biotechnology is needed, who makes final decisions and who takes responsibility.

V. Conclusion

Existing and developing genetic engineering (GE) technologies appear to be able to provide solutions to the problem of gene flow from GE and exotic plantation forests. However, there is little biological or social science research to inform about its impacts, the scale(s) at which it may become a concern, its significance in relation to other major sources of biodiversity perturbations, or the options for mitigation in the diverse species and coupled forest–human ecosystems where they might be applied. There are numerous opportunities for research to improve and extend GE technologies, and to fill gaps in knowledge of biodiversity impacts, but constraints in the form of market and regulatory restrictions to field research – and associated disinvestments in research from companies and governments – may make it difficult to fill these gaps in the foreseeable future.

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