

AN ABSTRACT OF THE THESIS OF

Stephen P. DiFazio for the degree of Doctor of Philosophy in Forest Science presented on January 7, 2002. Title: Measuring and Modeling Gene Flow from Hybrid Poplar Plantations: Implications for Transgenic Risk Assessment

Abstract approved: \_\_\_\_\_

Steven H. Strauss

Hybrid poplar plantations (*Populus trichocarpa* x *Populus deltoides*) are a relatively new feature on the landscape in the Pacific Northwest of the United States, and these plantations may soon include genetically engineered trees. Meanwhile, many wild poplar populations (*Populus spp.*) are highly degraded due in part to logging, dams, grazing, and agriculture. This raises the possibility that gene flow from plantation trees could have negative impacts on native tree populations. I analyzed gene flow from poplar plantations using a combination of large-scale field studies, genetic analyses, and simulation modeling. I describe development of a spatially explicit simulation model that depicts the processes of pollination, dispersal, establishment, competition, disturbance, and management activities on a landscape in western Oregon where hybrid poplar plantations are a prominent feature. Using sensitivity analyses, I demonstrate that competitiveness and fertility of transgenic trees were important factors determining the extent of modeled gene flow, and that these factors interacted such that the effects of enhanced competitiveness appeared to be obviated by cultivation of low-fertility transgenic trees. Disturbance regime, plantation silviculture, and the characteristics of the landscape surrounding plantations also had a strong influence on the rate of gene flow. Most modeled gene flow was due to long-distance transfer of pollen, and, to a lesser extent, seed. Modeled gene flow was insensitive to changes in vegetative dispersal and rates of vegetative establishment.

Field studies demonstrated low levels of gene flow from existing hybrid plantations in three settings. Gene flow was of a magnitude comparable to that achieved by the commonly cultivated horticultural variety Lombardy poplar (*Populus nigra* cv. *Italica*). I

conclude that gene flow to wild populations is low under existing conditions. There was a close match between observed and modeled pollen flow. However, seed flow and establishment were overestimated by the model due to conservative assumptions in a number of functions and parameters.

The model proved useful for examining scenarios of cultivation of transgenic trees. Trees containing a neutral transgene were predicted to constitute less than 1% of the basal area of wild poplar populations after 50 years. In contrast, an insect resistance transgene that conferred a substantial growth benefit in the wild resulted in a continual increase in transgenic trees in wild populations. This trend was dramatically slowed or eliminated for trees with reduced fertility. Finally, an herbicide resistance gene had little effect on overall modeled gene flow, but establishment was enhanced in a narrow subset of agricultural fields where the herbicide was used as a primary means of weed control. All of these model estimates were likely inflated due to consistently conservative assumptions about processes and parameters for which there was little available information.

Measuring and Modeling Gene Flow  
From Hybrid Poplar Plantations:  
Implications for Transgenic Risk Assessment

by  
Stephen P. DiFazio

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Stephen Paul DiFazio, Author

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*To my parents, my most important and enduring teachers*

# Measuring and Modeling Gene Flow from Hybrid Poplar Plantations: Implications for Transgenic Risk Assessment

## Chapter 1 Introduction

### **Background**

Humans have been altering the environment since prehistoric times by changing the composition of plant communities. Domestication of wild plants for agriculture began in Mesopotamia more than 10,000 years ago (Diamond 1997), and intentional introductions of exotic plants have occurred since at least 2500 BC, when Sumerians introduced species from Asia Minor (Gordon and Thomas 1997). Plant introductions are a double-edged sword. On the positive side, dissemination of domesticated plants and animals was an indispensable step in the development of human civilizations (Diamond 1997), and cultivation of exotic plants greatly enhances the quality of life in modern civilizations. However, scarcely a wild habitat exists today where exotic plants are not present, and in many cases these have had profound effects on native species and ecosystems (*e.g.*, (Ewel et al 1999; Williamson 1996).

Agriculture is perhaps the most dramatic and widespread form of plant introduction. For millennia plants have been bred and selected to provide food and other commodities, and these modified plants have been distributed throughout the world. Similarly, exotic trees have been used in intensive silvicultural systems for centuries, and they are a major source of fuel, wood, and pulp worldwide. The cultivation of hybrid varieties of poplar<sup>1</sup> has long been common practice in Europe, and is becoming increasingly important in this country. Poplars are the fastest growing commercial tree species in the United States (Cooper and Van Haverbeke 1990), with rotation lengths as short as 6 years for pulp production, and 8-15 years for lumber (Zsuffa et al 1996). In addition, facile vegetative propagation, ability to coppice, and broad ecological tolerances make poplar cultivation widely attractive (Wright 1994). Poplar cultivation has the potential to alleviate pressure

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<sup>1</sup> Throughout this dissertation, I will use 'poplar' to refer to members of the genus *Populus*, including aspens and cottonwoods.

for harvest of native forests through the conversion of relatively infertile or inaccessible agricultural lands into productive fiber and fuel producing areas (Dekker-Robertson and Libby 1998; Gladstone and Ledig 1990). Conversion of such lands could have other environmental benefits as well, such as reduced inputs of pollutants into aquatic systems, reduced erosion (Ranney and Mann 1994), enhanced wildlife habitat (Hanowski et al 1997), and sequestration of carbon to mitigate human-induced climate change (Intergovernmental Panel on Climate Change 2001).

The productivity of poplar plantations of the Pacific Northwest has been dramatically enhanced through the use of hybrids between the native black cottonwood<sup>2</sup> (*Populus trichocarpa* Hooker) and introduced eastern cottonwood (*Populus deltoides* Marshall.) (Stettler et al 1996). Also, the potential for further improvements through genetic engineering<sup>3</sup> is being actively pursued. Poplar has been transformed with traits such as herbicide resistance, insect resistance, altered lignin, and altered flowering (Strauss et al 2001). Poplar is particularly suited to genetic engineering because it is grown in intensive plantations in agricultural settings, so advances made in agricultural biotechnology can be readily transferred to poplar culture, and investments can be rapidly recouped (Sedjo 2001). Also, several characteristics of poplar biology make it particularly amenable to pioneering breakthroughs in biotechnology. First, poplar has a small genome size and a small ratio of physical-to-genetic map length, which facilitates isolation and cloning of genes (Bradshaw et al 2000). Second, poplar is readily regenerated in tissue culture and from vegetative cuttings, so genetic transformation and propagation is relatively straightforward (Han et al 1997). Third, poplar produces abundant seeds, and controlled crosses can be conducted on cut branches in greenhouses, thus allowing efficient generation of extremely large pedigrees (Stanton and Villar 1996). Fourth, poplar species occur over a broad range of environments and harbor a tremendous amount of genetic diversity. Many of these divergent poplar species are readily hybridized, which facilitates genetic mapping (Stettler, Zsuffa, and Wu 1996). Finally, there is a great deal of information about poplar biology, ecology, physiology, and genetics, and a large, collaborative research community is dedicated to the study of

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<sup>2</sup> 'Cottonwood' refers to species in sections *Aigeiros* and *Tacamahaca* of the *Populus* genus

<sup>3</sup> Genetic engineering is the introduction of genetic material by asexual means.

poplar biology (Bradshaw, Ceulemans, Davis, and Stettler 2000). In recognition of the potential of poplar as a model species, the United States Department of Energy will begin sequencing the entire poplar genome in 2002. Thus, poplar is poised to join *Arabidopsis*, the mouse, and the fruit fly as model organisms that propel biological knowledge forward.

Despite its great promise, the use of genetic engineering in forestry has raised significant concerns. The primary risks are that the tree itself may become more invasive, that competitiveness of wild relatives will be altered through transfer of the transgene<sup>4</sup> by hybridization, and that the transgene product or phenotype will have undesirable impacts on plantations, natural populations, or associated ecosystems (reviews in (James et al 1998; Mullin and Bertrand 1998). Of particular concern are traits such as herbicide and pest resistance, which may confer a fitness advantage and thus facilitate the spread of transgenic organisms (Ellstrand and Hoffman 1988; Regal 1994; Rogers and Parkes 1995). For instance, spread of an herbicide tolerance transgene in native poplar populations might cause a significant problem in conifer plantations where native poplars are considered weeds and herbicides are important for management (Strauss *et al.* 1997). Another major concern is that transgenic organisms could displace native trees in wild forests (Campbell 2000; Poppy 2000; Raffa 2001). This concern is not unique to transgenics, but extends to all plantings of exotic trees.

In the present study, we examined the potential for genes from plantations of hybrid poplar trees (*P. trichocarpa* x *P. deltoides*, TD) in the Pacific northwest to spread to native black cottonwood (*P. trichocarpa*) populations in western Oregon. Black cottonwood is widely distributed west of the Rocky Mountains, occurring from Baja California north along the coast, throughout the Cascade Mountains, and along the southern coast of Alaska (DeBell 1990). Through most of this range, cottonwoods are pioneers that are primarily restricted to riparian areas with high soil moisture and regular disturbance regimes (Braatne et al 1996; Mahoney and Rood 1998). They colonize river banks and sand bars that have been freshly scoured by flooding and which are continually moist (Baker 1990; Bradley and Smith 1986; Johnson 1994; Rood et al 1998).

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<sup>4</sup> A transgene is a gene introduced by genetic engineering. A transgenic organism contains DNA introduced by genetic engineering.

Cottonwoods are intolerant of competition, and little regeneration occurs beneath established vegetation (Braatne, Rood, and Heilman 1996; Schreiner 1945).

Cottonwoods are capable of extensive gene flow among populations via both pollen and seed. *P. trichocarpa* is a dioecious, wind-pollinated species that reaches sexual maturity in the wild at 6-10 years of age under permissive growth conditions. Flowering occurs in early spring and seeds are released in late spring. Seed crops are usually abundant, exceeding several million seeds per tree annually. Seed viability is initially very high, but seeds lose their viability within weeks under natural conditions (Schreiner 1974), and cottonwoods do not form persistent soil seed banks (DeBell 1990). The light and buoyant seeds with cotton-like appendages are capable of long-distance transport by wind and water (Johnson 1994). Low interpopulation differentiation at genetic marker loci, indicative of extensive gene flow, has been observed for several cottonwood species (Rajora et al 1991; Reed 1995; Weber and Stettler 1981).

In addition to high sexual fecundity, cottonwoods are also capable of vegetative propagation through root suckers, coppicing and formation of adventitious roots on abscised or broken branches (DeBell 1990; Gom and Rood 1999b). There is also evidence of cladogenesis, in which short shoots abscise and can be carried long distances on water courses and subsequently take root (Galloway and Worrall 1979). This mechanism potentiates long-distance vegetative dispersal, though it appears to occur at extremely low rates compared to pollen and seed dispersal (Galloway and Worrall 1979; Gom and Rood 1999a; Rood et al 1994).

Interspecific hybridization occurs commonly among natural populations of some *Populus* species. The genus *Populus* is divided into six sections, and species in the same and closely related sections are usually interfertile. Hybridization occurs freely between the sections *Tacamahaca* Spach, to which *P. trichocarpa* belongs, and *Aigeiros* Duby, to which *P. deltoides* belongs (Eckenwalder 1996). There are three species in section *Tacamahaca* in the U.S. (*P. trichocarpa*, *P. angustifolia* James and *P. balsamifera* L.) and two in section *Aigeiros* (*P. deltoides* and *P. fremontii* Wats.) (Eckenwalder 1984b). Of the 6 possible combinations of species in the two sections, five are sympatric and hybridize naturally ((Eckenwalder 1996; Rood et al 1986). In addition, many TD hybrids are interfertile with *P. trichocarpa* in greenhouse crosses (Chapter 3; (Bradshaw 1995).

Therefore, there is potential for extensive gene flow from TD plantations, which occur in close proximity to native *P. trichocarpa* populations.

## **Objectives and Organization of the Thesis**

The work described in this thesis is part of a project to assess risks of transfer of genetic material, particularly transgenes, from hybrid poplar plantations to wild populations. I describe an approach combining field studies of gene flow with a spatial simulation model that depicts spread of genes from plantations. The specific objectives were to:

1. Measure gene flow from hybrid poplar plantations,
2. Estimate fertility of hybrid poplar clones and interfertility with wild trees,
3. Study dispersal and establishment of seed and vegetative propagules from hybrid plantations,
4. Compare establishment rates and relative competitiveness of hybrid and wild seedlings,
5. Integrate the above information in a spatial simulation model,
6. Use the model to identify parameters most important in determining the extent of transgene spread,
7. Estimate potential gene flow from plantations under various scenarios of transgenic poplar cultivation, and
8. Evaluate the utility of the model for risk assessment of transgenic poplar cultivation.

Chapter two is an in-depth description of the spatial simulation model, the sources of data, assumptions implicit in the model design, description of functions and algorithms, and an exploration of the parameter space with sensitivity analysis. Chapter three describes our studies of gene flow from plantations and an assessment of the fit of model predictions to field observations. Chapter four describes application of the model to exploration of three scenarios: transgenic poplar with neutral fitness, herbicide-resistant poplar, and insect-resistant poplar. Chapter five presents a general summary and conclusions.

## **Chapter 2 A Spatial Simulation Model of Gene Flow from Transgenic Poplar Plantations**

### **Introduction**

Genetic engineering has the potential to revolutionize production of forest products and the practice of forestry. However, the great power of genetic engineering also raises the possibility of significant negative impacts on managed and natural ecosystems (Mullin and Bertrand 1998; Strauss *et al.* 2001a). Hazards associated with forest biotechnology will be highly trait- and environment-specific, and risk assessments must focus on these details (National Research Council 1989; Tiedje *et al.* 1989). However, some elements of risk are common to all genetic engineering, and in this respect a generic discussion of potential hazards is worthwhile.

One common element is gene flow, defined here as the establishment of transgenic organisms beyond the borders of plantations. Gene flow is a prerequisite for many potential ecological impacts of transgenic organisms (*e.g.*, (Mullin and Bertrand 1998b; Rogers and Parkes 1995; Snow *et al.* 1998). Some level of gene flow is likely for commercial-scale release of fertile transgenic trees with interfertile, sympatric wild relatives (Strauss *et al.* 1995). Therefore, many regulatory agencies may ignore the magnitude of gene flow and focus instead on potential hazards posed by transgenic organisms (D. Heron, USDA APHIS, personal communication). However, in some cases, such as localized field trials and cultivation of clones with reduced fertility, gene flow may fall below the threshold required for ecologically significant impacts (Levine 2000; Sakai *et al.* 2001). Therefore, an in-depth analysis of the dynamics and potential for gene flow from plantations will form an essential part of future risk assessments for a wide variety of traits and environments.

Trees present special challenges for quantifying gene flow. Long life spans and extended juvenile periods require studies of concomitantly long duration. Estimates of potential gene flow for transgenic herbaceous annual crops typically involve small-scale field experiments in which the crop is allowed to flower, seeds are collected from an array of receptor plants at various distances from the transgenic plot, and gene dispersal is estimated from the frequency of a marker genes at various distances (*e.g.*, (Arias and

Rieseberg 1994; Klinger et al 1992); (Kareiva et al 1994; Stallings et al 1995). Similarly, estimates of realized gene flow and relative competitiveness are usually derived from controlled experiments in which plants are followed for several generations and performance of transgenics is compared to conventional plants under a variety of conditions (*e.g.*, (Crawley et al 1993; Snow et al 1999; Snow et al 2001; Stewart et al 1997). Such methods are impractical for poplar, because 4-6 years would have to elapse from the time transgenic trees were planted until data on gene flow could be generated, multi-generation experiments would take decades to conduct, and the costs and logistics of transgene containment from tests of large, flowering trees are prohibitive. Also, because individual poplar trees may live for more than one hundred years, rare events could be important for reproductive success. For instance, natural poplar regeneration may depend on flooding that occurs at 10-year intervals or greater {Mahoney & Rood 1998 13 /id}1998). Such events would not be detected during most short-term field experiments. Also, large size and extensive gene flow distances necessitate studies that encompass very large areas (James, DiFazio, Brunner, and Strauss 1998). Therefore, methods are needed to extrapolate small-scale, short-term studies to appropriate temporal and spatial scales to allow transgenic risk assessment for trees (National Research Council 2000; Rogers and Parkes 1995; Snow 1999).

The problem of scale is not unique to transgenic risk assessment: it is a core issue in much of applied ecology (Levin 1992; Turner et al 2001). An emerging solution is the use of spatial simulation models to extrapolate results of short-term, small-extent field studies to relevant scales (Dunning et al 1995; King 1991). Here we describe a new spatial simulation model, STEVE (Simulation of Transgene Effects in a Variable Environment). The STEVE model depicts movement of transgenes from plantations, establishment and mortality of poplar cohorts, competition between genotypes within cohorts, management of plantations and surrounding lands, and perturbations such as herbicide spraying and insect attack in a spatially explicit landscape. The main purpose of STEVE is to explore the process of gene flow from transgenic plantations over long time frames and large land areas. The model is a tool for integrating the disparate sources of data in a logical framework, which in turn can be used to perform ‘virtual experiments’ to identify processes and parameters with the strongest influence on gene

flow. STEVE also allows exploration of a wide range of scenarios of cultivation of transgenic trees, from small-scale field trials with selectively neutral traits and reduced fertility, to large-scale commercial releases with advantageous traits and full fertility.

## **Methods**

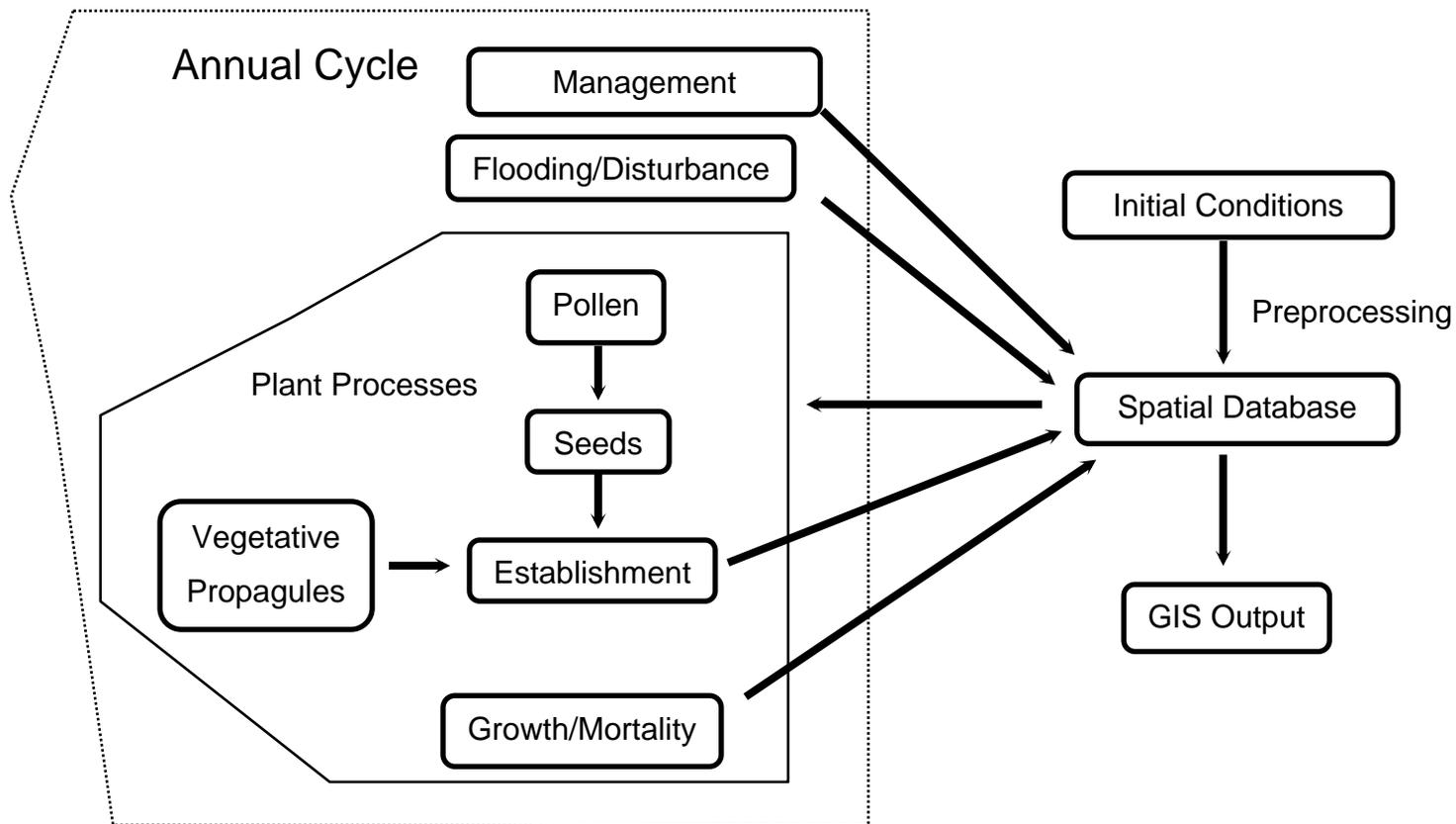
### **Overview of STEVE Model**

The main purpose of the STEVE model was to simulate movement of transgenes from poplar plantations. Our approach was to include the minimum number of variables and processes required to adequately represent this process. Therefore, all habitat definitions are based on the ability to transition to poplar, and all poplar populations are defined based on whether they were derived from transgenic or conventional trees.

The STEVE model operates on a landscape grid containing information about elevation, habitat type, and poplar populations. We derived landscape features by delineating habitat types from air photos (Allen 1999) and from a digital elevation model. We tessellated the landscape with a resolution of 0.1 ha cells (10 m x 10 m), which approximately represents the area occupied by a single large wild poplar tree. Cell sizes for forest landscape models are often in this range for the same reason (Higgins et al 1996; Shugart 1998). Each cell contains information about habitat type (Table 2.1), elevation, and spatial location relative to cells with similar characteristics. Also, all poplar trees within a cell are assumed to be of the same age (*i.e.*, a single cohort). The simulation has an annual time step, which begins with disturbance: the creation and conversion of poplar patches at rates determined empirically from a chronosequence of air photos (*e.g.*, (Jeltsch et al 1996; Turner 1987; Urban et al 1999)). Subsequently we simulate growth, reproduction, dispersal, and competition for poplar cohorts (Figure 2.1). Poplar behavior depends on location (plantation versus wild) and genotype (transgenic versus conventional). Basal area is proportional to age and number of trees, and pollen, seed, and vegetative propagule production are in turn proportional to basal area. This is all expressed on an arbitrary scale, because the main purpose of the model is to depict the

**Table 2.1** Description of habitat types in original data layers (1961, 1973, 1983, 1991) (Allen 1999; Cowardin et al 1979). Poplar habitat types below the double line were delineated for 1991 only, and encompass several of the broader habitat types. Habitat types were subsequently merged for the simulation (STEVE codes).

Habitat Type	Description	STEVE codes
Barren Land	Sand dunes, rock lands, sandy beaches, dredge spoils, and quarries	BARR
Agriculture	Field crops, pastures, orchards	AGRI
Urban	Residential, industrial, transportation, and mining operations	URBN
Forested wetland	Wetland with > 25% persistent trees	FWET
River	River	RIVR
Lake	Lake	WATR
Slough	Slough	WATR
Reed Canary Grass	Reed canary grass	WTLD
Estuarine Intertidal Wetland	Exposed wetland flooded by tides	WTLD
Lacustrine Tidal Wetland	Shallow wetlands (< 2 m) along lake shores	WTLD
Palustrine Wetland	Non-tidal wetlands with persistent emergents, shrubs, or trees (<25%)	FWET
Riverine Intertidal Wetland	Wetland adjacent to river, subject to periodic inundation and exposure	WTLD
Riverine Tidal Wetland	Wetland adjacent to river, low gradient, water velocity influenced by tides	WTLD
Shrub/Scrub	Burned areas, recent clearcuts	SCRB
Grassland	Grassland	SCRB
Mature Coniferous	Coniferous forest, > 5 m height	MFOR
Young Coniferous	Coniferous forest, < 5 m height	IFOR
Mature Broadleaf Forest	Broadleaf forest in uplands, > 5 m height	MFOR
Young Broadleaf Forest	Broadleaf forest in uplands, < 5 m height	IFOR
Mature Mixed Forest	Mixed conifer and broadleaf forest in uplands, > 5 m height	MFOR
Young Mixed Forest	Mixed conifer and broadleaf forest in uplands, < 5 m height	IFOR
Poplar Plantation	Plantation	PLNT
Young Mixed poplar	Up to 70% poplar, < 5 m height	CTWD
Mature Mixed poplar	Up to 70% poplar, > 5 m height	CTWD
Young Pure poplar	Greater than 70% poplar, < 5 m height	CTWD
Mature Pure poplar	Greater than 70% poplar, > 5 m height	CTWD



**Figure 2.1** Flow diagram for STEVE model. Model begins with preprocessing of GIS layers representing initial simulation conditions. Data are stored in a spatial database containing information about elevation, cover type, poplar populations, plantations, and agricultural fields. Simulation begins with management activities such as plantation harvesting and herbicide spraying. Poplar establishment and mortality is simulated in the disturbance function. Seed, pollen, and vegetative propagules are produced proportional to basal area of each genotype, followed by dispersal, establishment, growth and mortality. Outputs are text files and spatial data layers.

relative balance between transgenic and conventional trees, so processes are driven by the proportion of each genotype rather than absolute numbers.

Pollen and seed are produced in each cell proportional to basal area of each genotype, modulated by a fecundity factor. Dispersal occurs explicitly within local ‘neighborhoods’ according to functions derived from extensive field studies (unpublished data). Also, pollen and seed production of each genotype are tallied for the whole landscape, and a proportion of seeds and seedlings are derived from a ‘background’ cloud of pollen and seeds, also calibrated based on field studies. Vegetative propagules are likewise produced in proportion to basal area and dispersed locally.

Establishment occurs only in sites previously cleared by ‘disturbance.’ We assume that adequate propagules will be available to occupy all available establishment sites, though the number of established seedlings is varied stochastically to accommodate variation in propagule abundance and interspecific competition. Different establishment rates are allowed for seeds produced locally, seeds from the ‘background’, and vegetative propagules, and establishment of each genotype depends on relative abundance within each propagule type.

All previously established cohorts then undergo density-dependent mortality, modulated by an age-dependent carrying capacity and a competitive differential for transgenics. This competitive differential can be positive, negative, or neutral, and this is the primary means for simulating fitness effects of different transgenes. Poplar patches are not eliminated by density-dependent mortality: it is a means for simulating intraspecific competition between genotypes. Cells must contain at least one tree following this process. Poplar patches are only eliminated by the disturbance function (above).

Finally, management activities are carried out in poplar plantations and agricultural fields. As is the prevailing management practice, poplar plantations occur as large monoclonal blocks (mean = 16 ha). Harvesting occurs when plantations surpass rotation age, and new plantations are established according to predetermined rates of establishment of transgenic, conventional, male, and female clones. Agricultural fields contain a variety of crops defined broadly based on management characteristics (annual versus perennial, tilling, grazing, herbicide treatments). Agricultural fields are also

abandoned or established according to empirically determined rates, and abandoned fields become available for colonization by poplar in subsequent years.

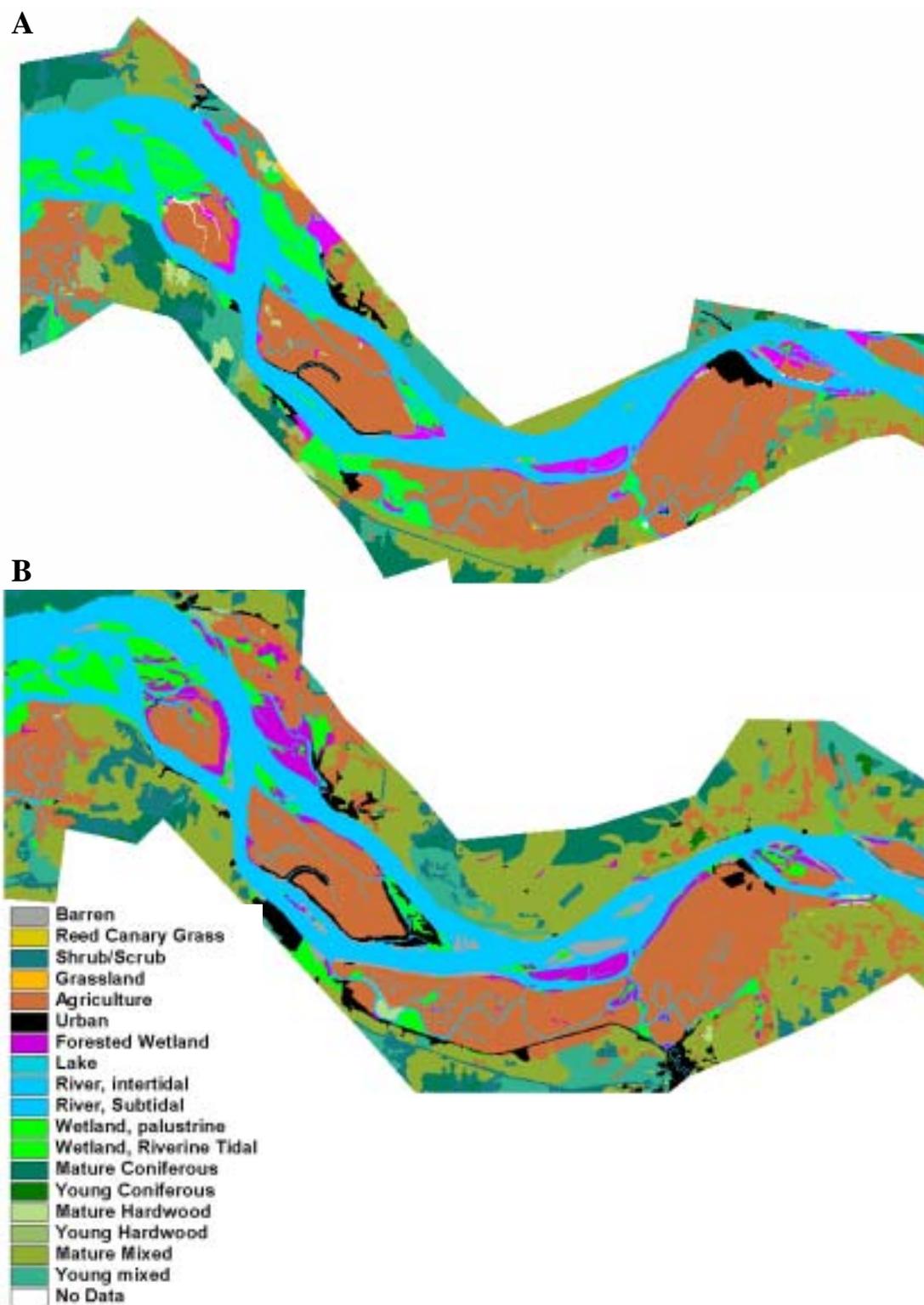
## **Landscape Data**

### *Primary Data*

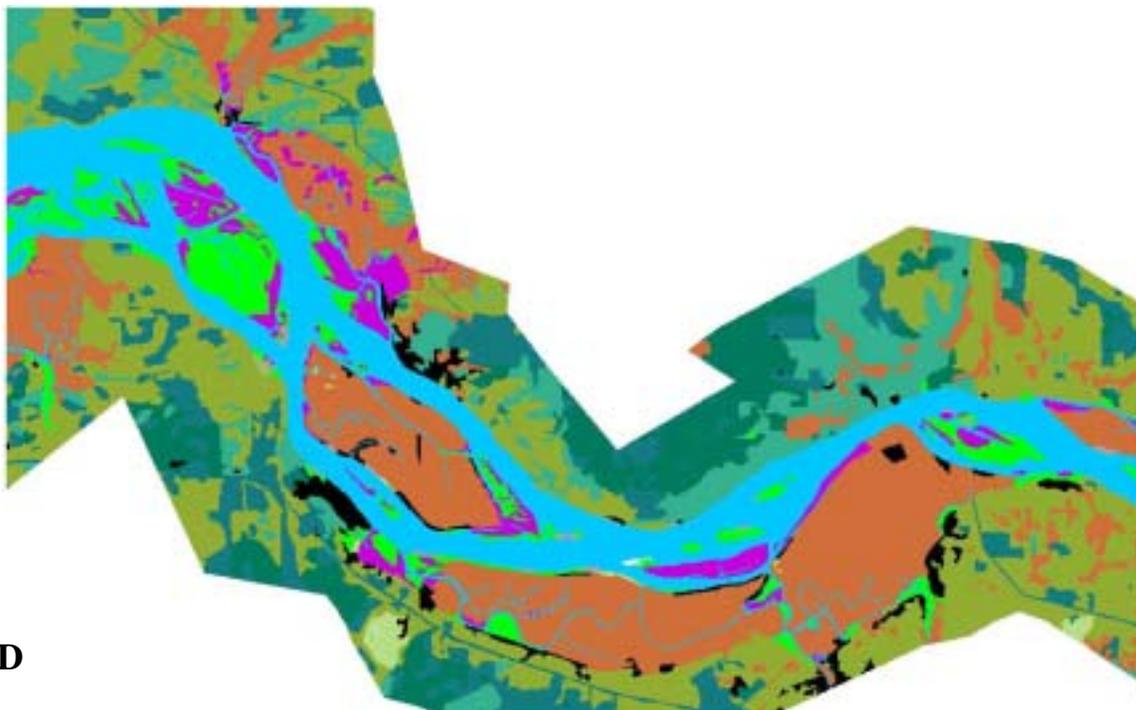
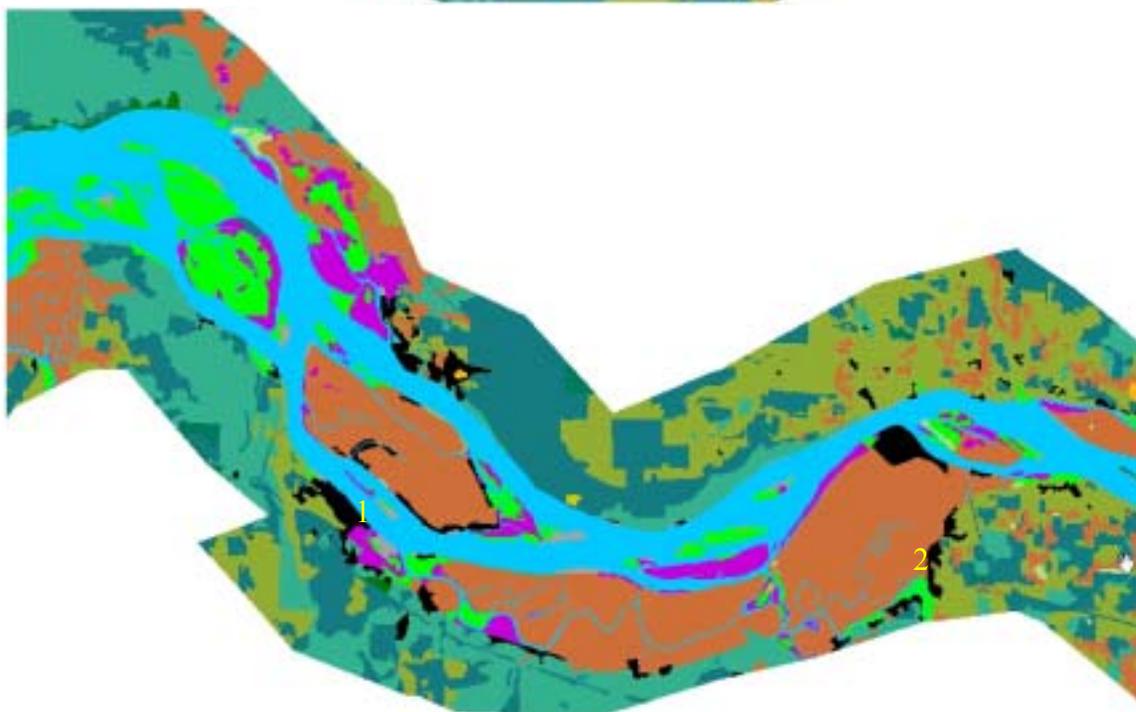
Habitat types were delineated as part of a joint project between Oregon State University and the U.S. Army Corps of Engineers aimed at assessing changes in wetland habitats since 1948 on the lower Columbia River (Allen 1999). For the present study, we used data from river km 49 to 93, encompassing a strip 5 km wide on both sides of the Columbia River. This is currently an area of intensive poplar cultivation. Air photos were used to delineate broad habitat types in the study area (Table 2.1). Photos were primarily black and white and 1:48,000 in scale. Photos were laid out in adjacent flight lines, overlaid with mylar, and viewed with a stereoscope. Habitats were delineated on the mylar and subsequently zoom-transferred to United States Geological Survey 7.5' quadrangle maps to correct for variation in scale and aerial distortion. Habitats were classified in this manner for 1961, 1973, 1983, and 1991 (Figure 2.2).

The data were supplemented and adapted for the current project by delineating poplar stands from 1991 photos only. Poplar trees were distinguished from other hardwoods based primarily on geomorphology, crown structure, and size. Poplars occur as linear or arcuate features, primarily within the active and historic flood plain, most often on well-drained soils on riverbanks, bars, and islands, though they can occur on upland sites if appropriate moisture conditions exist (Braatne et al 1996). In addition, crown margins have a rounded shape and a clumped foliage pattern, and poplar crowns are often emergent in mixed stands (DeBell 1990).

Poplar stands were divided into 'pure' (>70% cottonwood) and 'mixed' stands, and 'mature' and 'immature' size classes (< 5 m height). Generally, only stands of 0.5 ha or more were identified, though smaller patches could be delineated if they occurred in diagnostic shapes and locations (*e.g.*, linear stands on river banks). Accuracy was improved in key areas (*e.g.*, in the vicinity of poplar plantations) by using larger scale



**Figure 2.2** Habitat types on lower Columbia River between Westport (1) and Clatskanie (2) Oregon. **A.** 1961. **B.** 1973. **C.** 1983. **D.** 1991.

**Figure 2.2 (Continued)****C****D**

color photos. In addition, data were verified and corrected following multiple field visits using a hand-held Global Positioning System unit.

The mylar overlays were digitized and polygon topology was built and cleaned using Arc/Info 7.2 (ESRI Inc., Redlands, CA). This vector layer was then converted to a grid file with 10 m cells (Figure 2.3A). We derived layers depicting distance and direction to the river for each cell. In addition, we obtained a 33 m digital elevation model for the study area and resampled this to a 10 m grid size and derived slope and aspect using ARC/Info (Figure 2.3B).

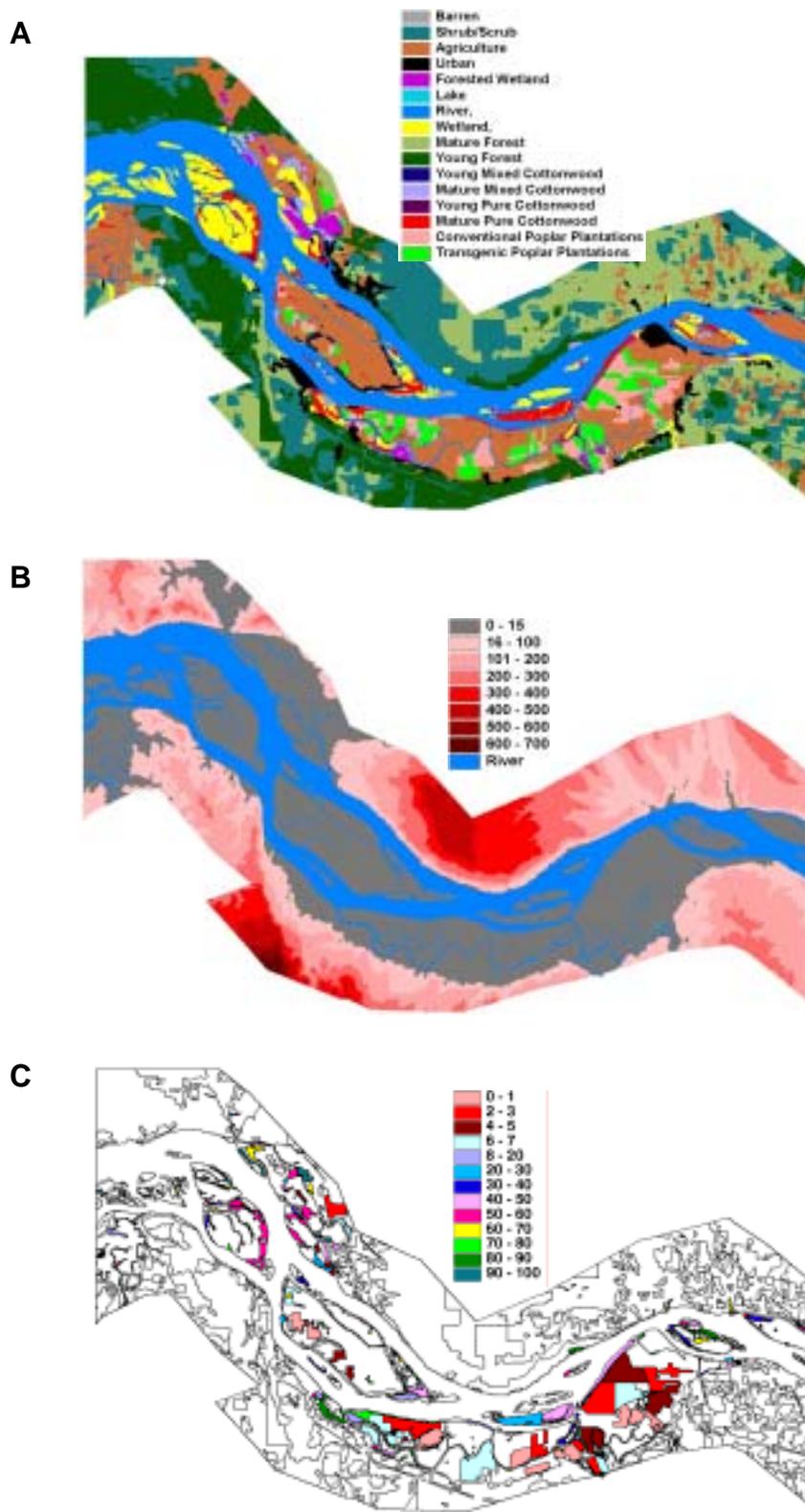
All grid layers were exported as ASCII files containing a single datum for each cell of the grid. These ascii files were subsequently converted to binary files to be used for the simulation.

### *Implementation in Model*

We created a preprocessor to organize the data from the binary layers into a logical format (data structures) to maximize efficiency of the simulation. The preprocessor creates new binary files (a ‘habitat’ file and a ‘poplar’ file) with the information organized into structures. Other variables are set each time the model is run, which allows customization of runs. There are five main data structures associated with landscape features (Table 2.2) :

- a ‘Landscape’ structure that defines location on the landscape (essentially a place holder),
- a ‘Habitat’ structure that defines habitat type, elevation, and polygon membership,
- a ‘Poplar’ structure that defines poplar characteristics,
- an ‘Establish’ structure that accumulates propagule data in establishment sites (this disappears following establishment), and
- an ‘Ag’ structure that defines agricultural fields.

These structures are linked to one another within a cell, and to structures in adjacent cells, to facilitate rapid processing.



**Figure 2.3** Starting landscape conditions. **A.** Cover type. **B.** Elevation.. **C.** Age of poplar stands and plantations.

### Wild Poplar Stands

Our information on poplar stands was approximate, having been derived principally from low-resolution air photos. We therefore created simulated populations based on localized observations as described below. Initially all trees outside plantations were nontransgenic.

#### Age

We assigned age randomly between 1 and 10 for ‘immature’ stands (those less than 5 m tall) and between 10 and 100 for mature stands (Figure 2.3C).

#### Density

We first derived a relationship between carrying capacity and age (Figure 2.4), based in part on data from experimental research plots (DiFazio *et al.*, 1999):

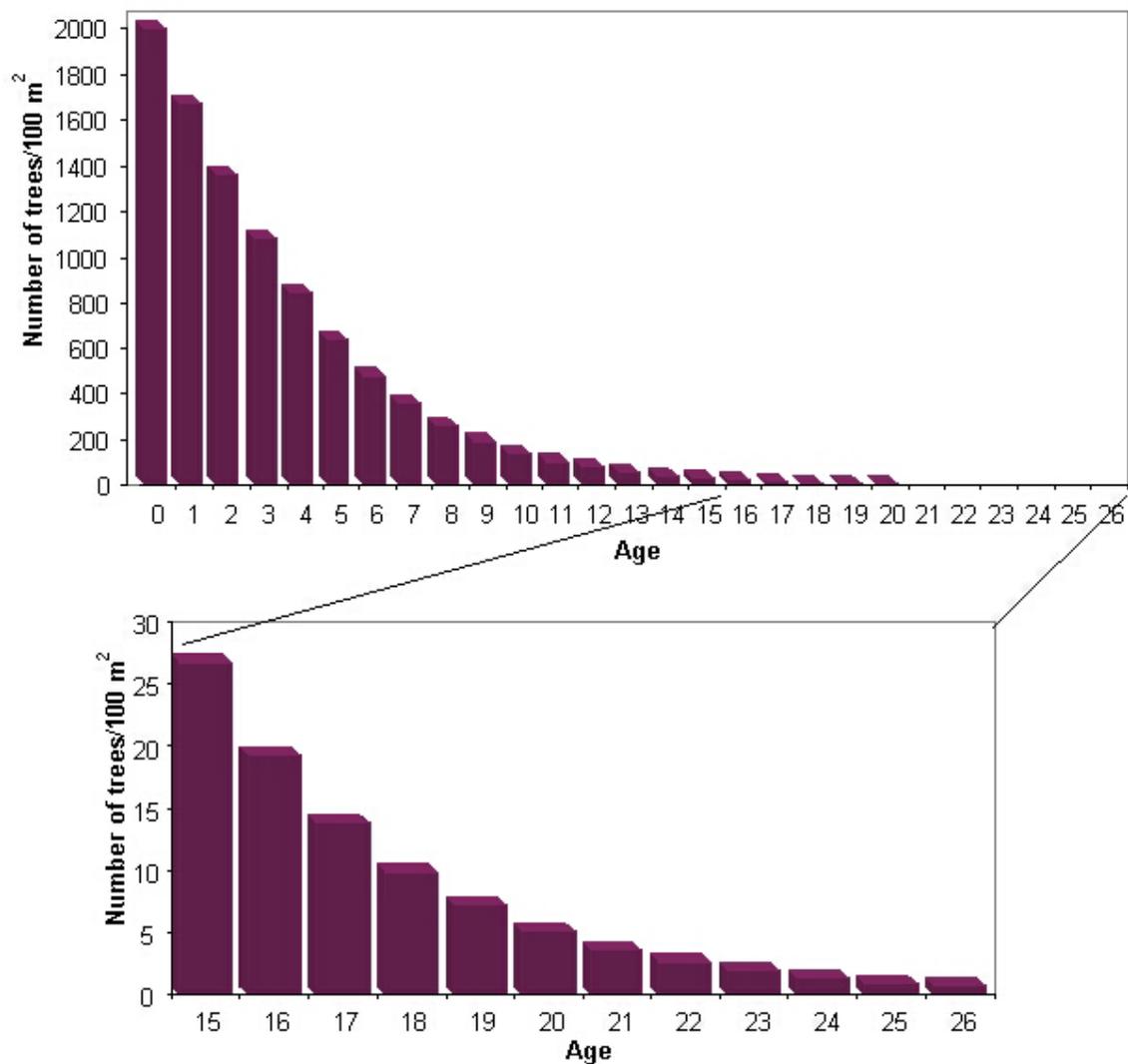
$$N_{max_a} = 1 + 4000 * \left( \frac{e^{-\frac{a}{3}}}{1 + e^{-\frac{a}{3}}} \right)$$

where  $a$  is age.

For pure stands, the initial number of trees per block was randomly assigned between 70 and 100% of carrying capacity for that age. For mixed stands, initial density was randomly set up to 70% of carrying capacity. This was based on the criteria used in delineating poplar stands from air photos.

#### Basal Area

Basal area is related to age, relative density, and genotype. It is a saturating exponential function that maximizes at 1 (a relative, unitless value) (Figure 2.5). Annual increment begins to slow before 50 years, which is in the range of values observed for ‘maturity’ of black cottonwood in the Pacific northwest (DeBell 1990). The equation is:



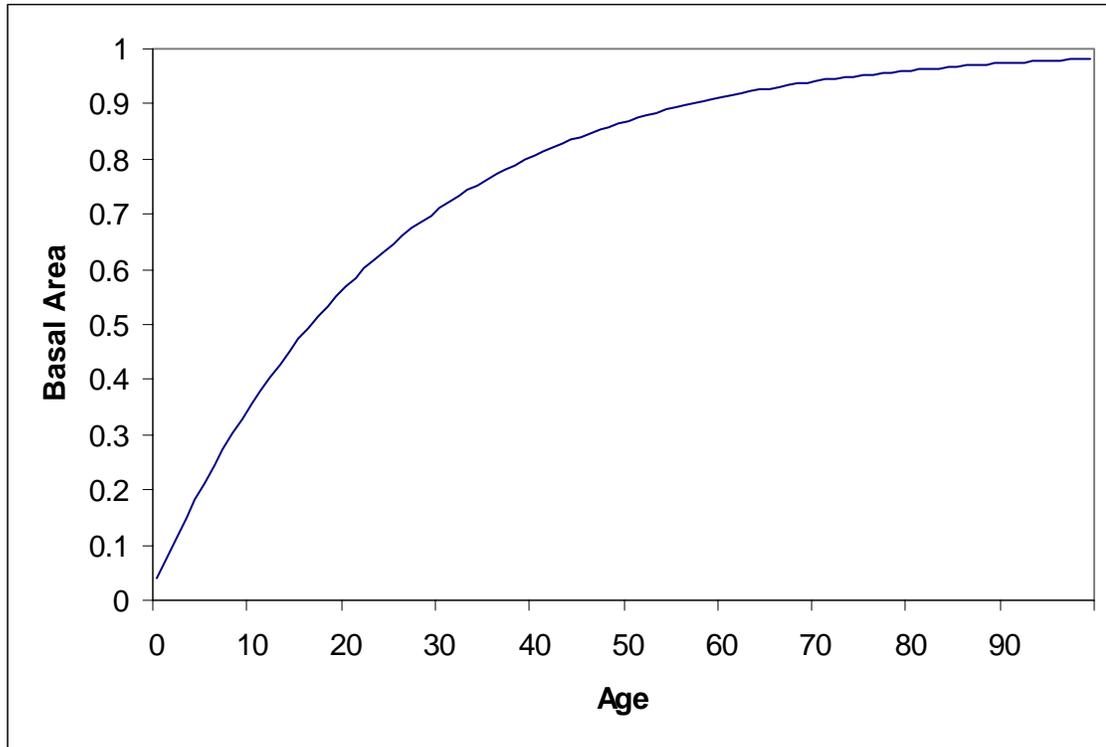
**Figure 2.4** Calculation of poplar carrying capacity ( $n_{max}$ ) of a 100 m<sup>2</sup> area as a function of age. The equation was derived from empirical observations of experimental plots up to age 3, and the assumption that a single tree will occupy the area by age 25.

**Table 2.2** Organization of data on landscape and poplar characteristics

<b>Landscape Data</b>	Information on spatial location: row and column;
<b>Habitat Data</b>	Information related to physical habitat
Cover	Habitat type (table 2.1)
Elevation	Elevation above sea level (m)
Agriculture	Set for agricultural fields only (Table 2.3)
Establishment	Indicates if habitat is open for establishment (transient)
Edge	Indicates if habitat is at edge of poplar polygon
Polygon	A unique identifier for each patch
<b>Poplar Data</b>	Characteristics of cohorts of poplar trees
Density	Number of transgenic and conventional (t and c) trees
sex ratio	Proportion of cohort that is male (t and c)
basal area	A corollary of mean tree size (t and c)
Fecundity	Multiplier for seed and pollen production (t and c)
Age	Number of years since establishment
Phenology	Flowering time (broad classes)
Herbivory	Presence or absence of defoliating insects
pollen production	Annual pollen production (t and c)
branch production	Annual branch production (t and c)
shoot production	Annual shoot production (t and c)
pollen input	Counter for pollen input from surrounding cells (t and c)

$$Ba_{ga} = \alpha \frac{N_g t (1 - e^{-0.04a})}{N \max_a}$$

where  $\alpha$  is the proportional difference in size (or resource acquisition) between transgenic and conventional trees,  $N_g$  is the number of trees of genotype  $g$  (transgenic or conventional),  $a$  is age,  $N \max_a$  is the carrying capacity at age  $a$ , and  $t$  is time.



**Figure 2.5** Maximum basal area versus age. Basal area scales linearly with density and is related to age with a saturating exponential function.

### Sex Ratio

Sex ratio was determined through a binomial sampling process. Each tree in a cohort was initially assigned sex with a 50% probability of being male or female.

### Floral Phenology

Flowering phenology parameters are set at the time of initialization. The user sets the number of phenology classes, a compatibility parameter, and the distribution of plantation and wild trees among classes. See the pollination function for further explanation of the representation of flowering phenology.

### Poplar Plantations

Poplar plantation characteristics are set from a configuration file at the time of model initiation. Plantations are divided into blocks or management units, each of which may be planted with a different clone or genotype (transgenic or conventional). Each block is

assigned an age, sex, and genotype (transgenic or conventional) with the configuration file. Floral phenology is set randomly at rates determined by another input file. Values for plantations established after model initiation (*i.e.*, following harvest) are set randomly according to probabilities set by the user. Plantation density, rotation, and age of flowering can all be customized for each model run.

### Agricultural Fields

Key characteristics can be individually customized for agricultural fields at model initiation. Each field is assigned a generic crop and key associated characteristics (Table 2.3): herbicide usage (one target herbicide), annual tilling, and grazing. In addition, a year is set at which the field is abandoned, at which point it becomes available for conversion to poplar forest based on empirical transition rates (see Disturbance, below). Poplar forests are converted to new fields containing the different crops at rates set by the user. Edges of agricultural fields are identified and assigned a separate habitat type to allow stochastic establishment of volunteer poplar trees.

### *Assumptions and Limitations*

1. *Poplar establishment and stand development occur as an even-aged cohort on a 100 m<sup>2</sup> scale or greater.* This is a reasonable assumption because of the pioneer habit and shade intolerance of this species (Braatne, Rood, and Heilman 1996). Areas of establishment might be smaller than 100 m<sup>2</sup>, but for the purpose of simplicity we are simulating this by randomly varying the number of trees establishing. Certainly vegetative reproduction is common within stands, so they can appear to be of mixed ages as they develop. However, from a population ecology perspective it is reasonable to treat vegetative reproduction as growth of a genet (Harper 1977), which is our approach here.
2. *Maximum stand age is initially 100 years.* Although individual poplar trees may live longer than 100 years, we believe it would be unusual for a large cohort of ramets to survive that long in this dynamic landscape. Approximately 40% of the poplar stands in the study area were converted to some other habitat type between

1961 and 1991 (see Disturbance section below), and most stands in the study area appeared relatively young.

3. *Sex ratio is 1:1 on landscape.* There have been numerous reports of variation in sex ratio of poplar, and hypotheses about differential selection of the sexes (*e.g.*, (Farmer, Jr. 1964; Noh et al 1988; Rottenberg et al 2000). However, sex ratio has consistently been found to be 1:1 when viewed at sufficiently large scales (Farmer, Jr. 1964; Noh, Lee, Koo, and Chung 1988), and our binomial sampling method and mortality algorithm (below) allow for locally skewed sex ratios.

**Table 2.3** Crop characteristics. Herbicide is the annual probability of spraying a particular herbicide (*e.g.*, glyphosate). Till is the probability of annual tilling. Graze is the probability of regular grazing by livestock.

Crop	Herbicide	Till	Graze
Row Crop	0.5	1	0
Pasture	0.01	0	1
Grass Seed	0.01	1	0
Tree Farm	0.5	0	0

## Disturbance

### *Primary Data*

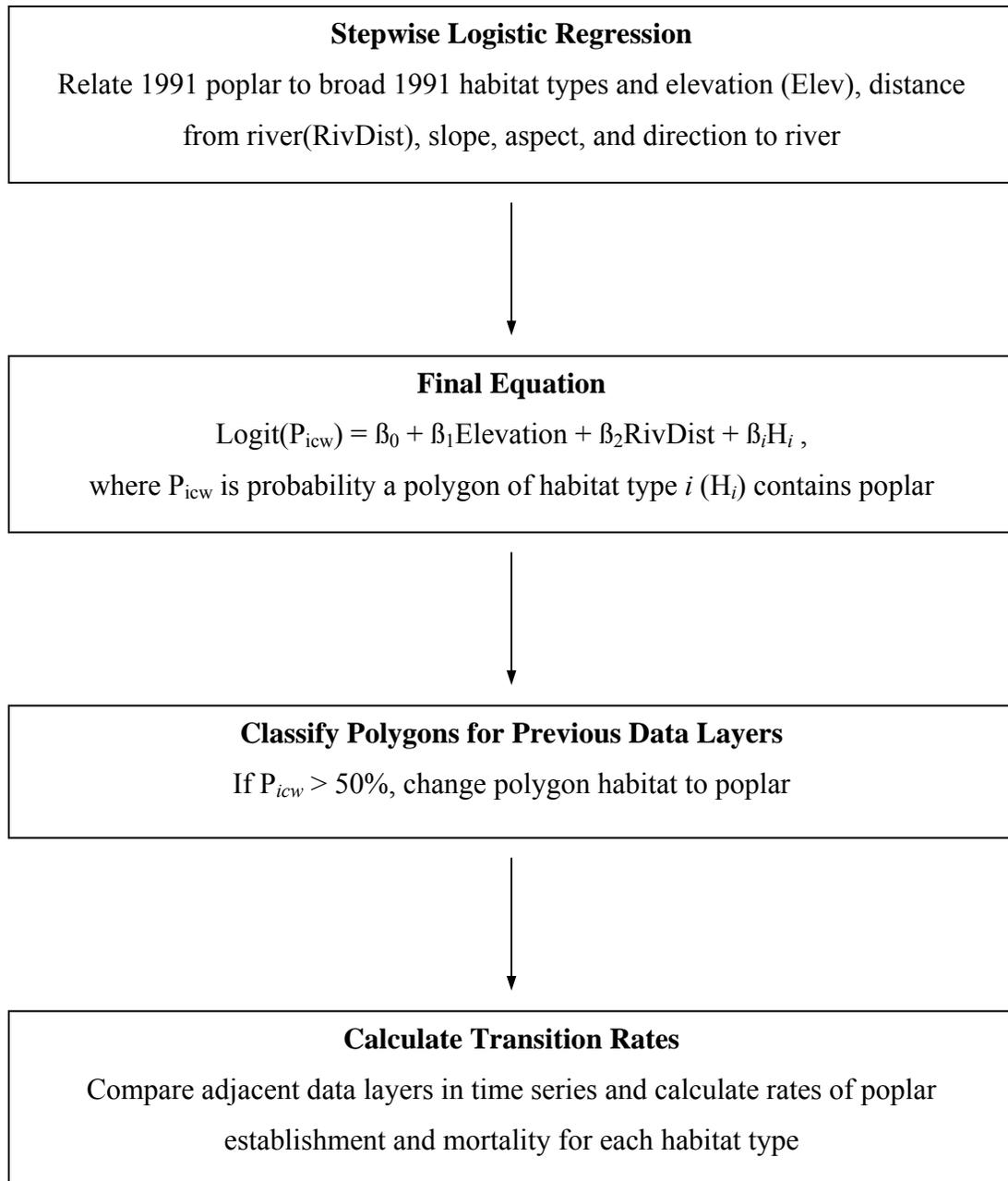
We modeled change in poplar stands using a chronosequence approach, a method that has been applied extensively in analyses of landscape change (*e.g.*, Frelich *et al.* 1993; (Turner 1987). The general concept is that changes in delineated poplar polygons from different dates reflect establishment and mortality of poplar stands. Due to financial and time constraints, we were only able to explicitly delineate poplar stands for the 1991 time point. Habitat types were defined much more broadly for previous years (Figure 2.2), and habitat types such as forested wetland, palustrine wetland, broadleaf forest, and mixed forest, all contained poplar trees to varying degrees. Therefore, we used the relationship between the original habitat types, environmental variables, and the occurrence of cottonwood in 1991, to derive a predictive equation that could be applied to earlier data layers (Figure 2.6).

The predictive equation was derived using stepwise logistic regression. Explanatory variables were habitat type, elevation, slope, aspect, distance to river, and direction to river. Elevation and distance to river were both negatively associated with occurrence of poplar, and the habitat types Barren, Forested Wetland, and Mature Broadleaf were positively associated with cottonwood, and Riverine Tidal Wetland was negatively associated with cottonwood (Tables 2.1 and 2.4).

We assessed the predictive power of the logistic regression by comparing the observed distribution of poplar for 1991 to the predicted distribution, using different cutoffs for predicted probabilities (Figure 2.7A). We determined that a cutoff of 49% for assigning a polygon to poplar gave the highest success rate (overall correct classification rate = 98%, Cohen's Kappa = 0.70,  $Z = 414.33$ ,  $P < 0.001$ ).

We used this equation and probability cutoff to map poplar onto the broad habitat designations of 1961, 1973, and 1983 data layers. To assess the accuracy of this assignment, we obtained a subset of the air photos that were used in the original air photo survey. These photos were at a scale of 1:24000 for 1961 and 1973, and 1:48000 for 1983. They covered a total of 630 ha (1961), 1107 ha (1973), and 3426 ha (1983). We delineated poplar in these selected regions and assessed correspondence to predictions from the logistic regression equation derived from 1991 data. There was a close match for 1961 and 1973, but the logistic regression overpredicted 1983 poplar stands (Figure 2.7B). Part of this discrepancy may have been due to poor quality of air photos for 1983, resulting in failure to delineate some stands.

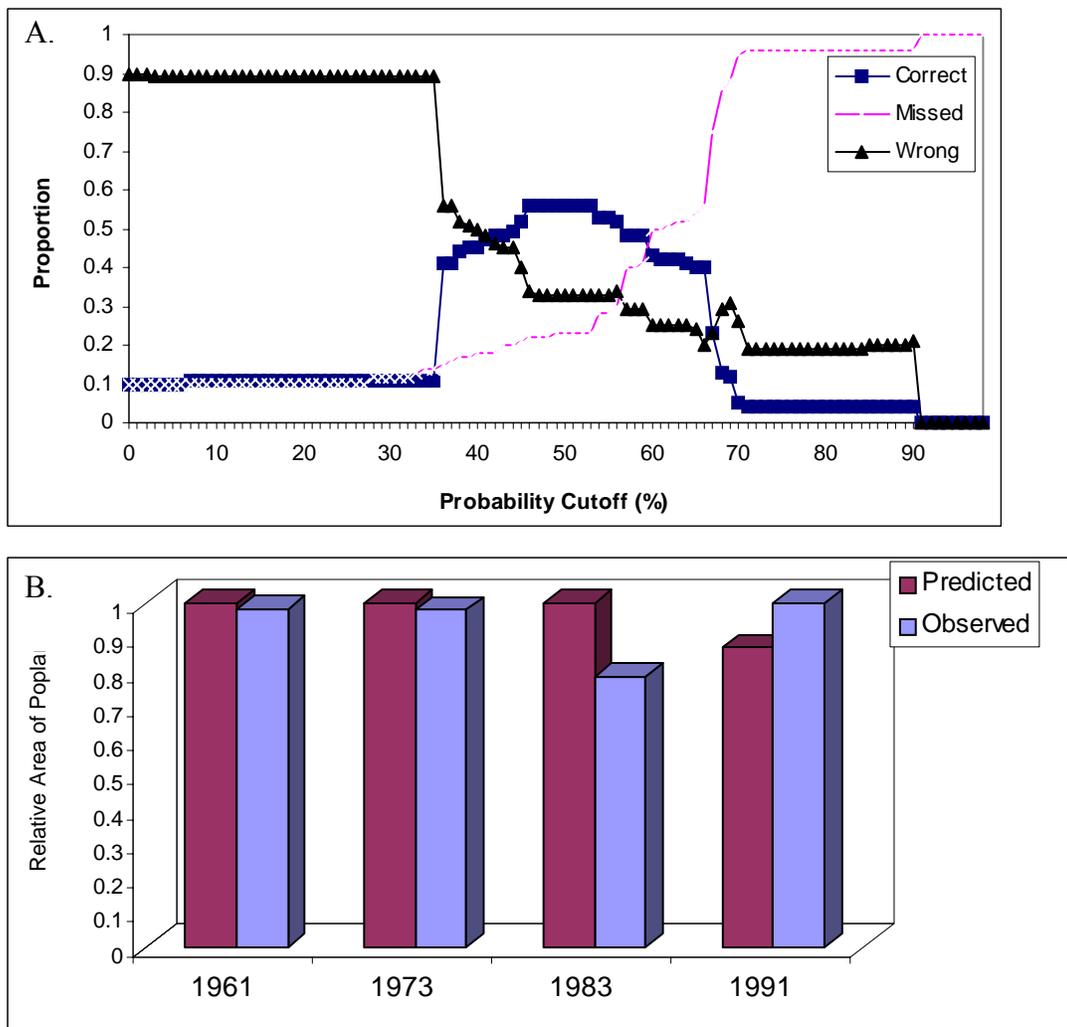
These reclassified layers containing poplar designations were then used to calculate transition rates between poplar and other habitat types by overlaying layers of adjacent years and tallying changes in habitat types of intersecting polygons. We chose to focus on two key transition periods: 1961 to 1973, and 1983 to 1991. The earlier transition represents the prevailing disturbance regime before the major flood control dams were instituted (*i.e.*, the John Day dam, 1968), and at least one large flood occurred in 1964 (Allen 1999). The later transition period represents the post flood-control regime, though some residual effects of earlier floods might still be evident.



**Figure 2.6** Method for calculating rates for poplar establishment and mortality from a chronosequence of air photos. All photos in the series were classified using broad habitat types (Table 2.1), and the 1991 layer was also classified for the presence of cottonwood. We used the relationship between the broad habitat types, environmental variables, and occurrence of cottonwood in 1991 to classify previous data layers for cottonwood occurrence. We then calculated ‘transition’ rates between cottonwood and other habitat types for adjacent data layers (*i.e.*, 1963 to 1971 for pre-flood control rates and 1983 to 1991 for post-flood control rates).

**Table 2.4** Results of logistic regression analysis for classifying 1991 polygons (N=937) for probability of containing poplar (N=250). Elevation and distance from the river entered the model following a stepwise selection procedure. SE, Standard Error. Significant parameters are highlighted in bold.

Variable	DF	Parameter Estimate	SE	Wald Chi-Square	Pr > Chi-Square	Odds Ratio
Intercept	1	-0.0943	0.2155	0.1912	0.6619	.
<b>Barren</b>	1	-3.4693	1.0321	11.2983	0.0008	0.031
<b>Forested Wetland</b>	1	1.0158	0.2428	17.5038	0.0001	2.762
Lake	1	-14.2156	944.3	0.0002	0.9880	0.000
River	1	-14.7924	622.9	0.0006	0.9811	0.000
Slough	1	-0.4410	0.7406	0.3547	0.5515	0.643
Reed Canary Grass	1	-14.5858	1236.1	0.0001	0.9906	0.000
Palustrine Wetland	1	0.00973	0.2598	0.0014	0.9701	1.010
<b>Riverine Tidal Wetland</b>	1	-2.3597	0.6322	13.9322	0.0002	0.094
Shrub	1	-11.2615	157.1	0.0051	0.9428	0.000
Grass	1	-3.8244	1519.6	0.0000	0.9980	0.022
Mature Conifer	1	-3.4103	770.8	0.0000	0.9965	0.033
Immature Conifer	1	-10.7137	1619.5	0.0000	0.9947	0.000
<b>Mature Broadleaf</b>	1	2.5363	1.0710	5.6086	0.0179	12.633
Immature Broadleaf	1	0.4744	1.4324	0.1097	0.7405	1.607
Mature Mixed	1	1.1329	0.7688	2.1713	0.1406	3.105
Immature Mixed	1	-0.4491	0.8301	0.2927	0.5885	0.638
<b>Elevation</b>	1	-0.0983	0.0248	15.6880	0.0001	0.906
<b>Distance from River</b>	1	-0.00076	0.00036	4.3894	0.0362	0.999



**Figure 2.7 A.** Performance of logistic regression in predicting poplar occurrence in 1991. Probability cutoff is the threshold probability for designating a polygon as poplar. ‘Correct’ polygons are poplar polygons with probabilities greater than the cutoff value. ‘Missed’ polygons are poplar polygons that had probabilities lower than the cutoff value. ‘Wrong’ polygons are non-poplar polygons with probabilities greater than the cutoff value. The optimal cutoff was determined to be 49-53%, because this is the value at which correct designations are maximized and errors are minimized. Therefore, all polygons with predicted probabilities of 49% or greater were designated as poplar for data layers that were characterized for broad habitat types only. **B.** Observed versus predicted occurrence of poplar for selected regions of landscape. Predicted area was delineated by applying a logistic regression model to broad habitat types and environmental data from each year. Observed values for 1961, 1973, and 1983 are derived from an analysis of airphotos encompassing 630 ha, 1107 ha, and 3426 ha, respectively. Data for 1991 were derived from the entire landscape (24,207 ha).

An initial analysis of transitions revealed that for several habitat types there were very few observations available, so we merged a number of habitat types into broader classes on the assumption that these habitat types would behave similarly in transitions to and from poplar (Table 2.1). A logistic regression analysis of poplar transition revealed that none of the merged original habitat types were significantly associated with cottonwood establishment, and elevation appeared to be the main driver (not shown). Virtually no poplar occurred above 15 m elevation at the study area, so elevations higher than 15 m are excluded from further consideration. Annual poplar establishment rates (Figure 2.8A) were calculated as:

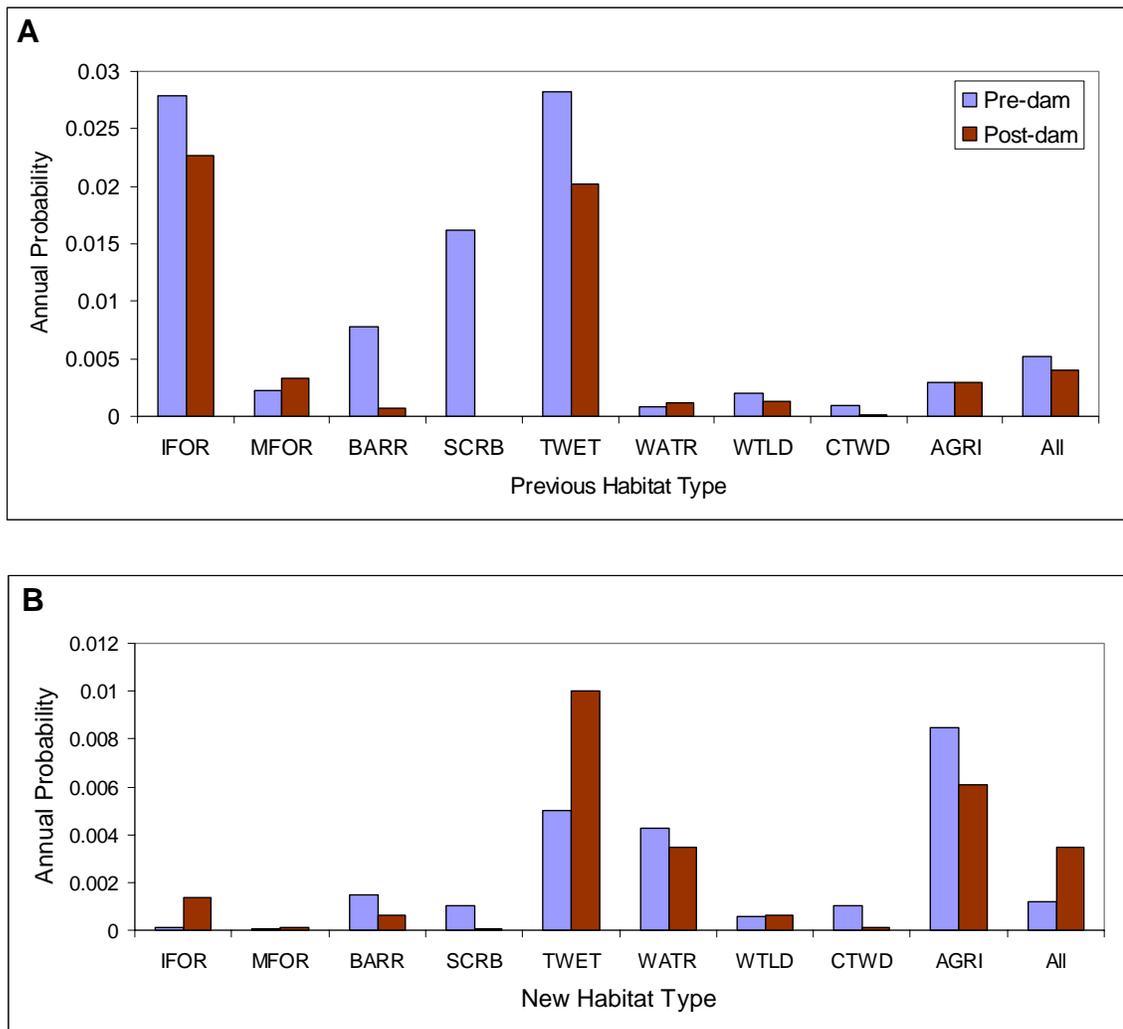
$$E_h = \frac{A_{ph}}{A_h t}$$

where  $A_{ph}$  is area (number of cells) of habitat type  $h$  in prior data layer that became poplar during the interval,  $A_h$  is total area of habitat type  $h$  in the previous data layer for which data exist in the more recent data layer, and  $t$  is number of years in the interval. Similarly, mortality rates were calculated as:

$$M_h = \frac{A_{hp}}{A_p t}$$

where  $A_{hp}$  is area of poplar that became habitat type  $h$  during the interval, and  $A_p$  is total area of poplar (Figure 2.8B).

In addition to transition rates, we also determined the distribution of establishment and mortality patch sizes for each interval (Figure 2.9).



**Figure 2.8** Probabilities of poplar establishment and mortality, as calculated from transition rates from 1961 - 1973 data layers (pre-dam) and 1983 - 1991 (post-dam). **A.** Establishment rates. **B.** Mortality rates. See table 2.1 for abbreviations.

### *Implementation in Model*

The first step in simulating creation and destruction of poplar populations is to select the disturbance regime. The main factor controlling disturbance in this system is flooding, and this is greatly attenuated by flood-control dams. However, as demonstrated in 1996, large floods are still possible in this system, and we assume these will occur at approximately 100-year intervals. Therefore, we instituted the pre-flood control disturbance regime (*i.e.*, 1961-1973 transition rates) with a 1% probability.

The probabilities calculated above are on an areal basis, and therefore represent the probability that a given unit of land (a cell) will be converted. However, disturbances are generally larger than one cell (100 m<sup>2</sup>) in size (Figure 2.9A-D), so multiple cells must be converted in unison. Our approach is to first calculate the total area (number of cells) to be converted in a given year. For each habitat type  $h$ , area converted to poplar annually is:

$$C_{hp} = E_h A_h$$

Similarly, area of poplar to be converted to each habitat type is:

$$C_{ph} = M_h A_p.$$

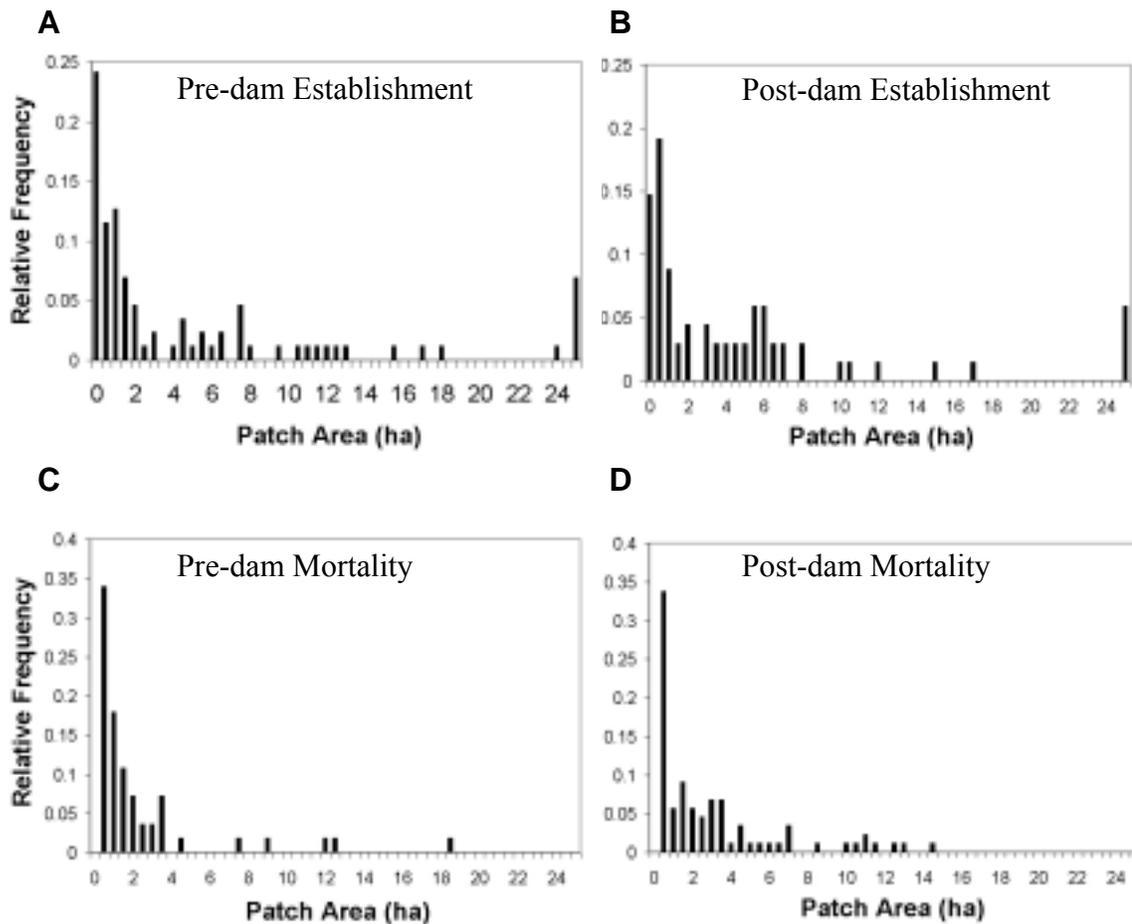
Then we sample polygons from the empirical distributions of patch sizes (Figure 2.9A-D) until the total area of the polygons exceeds the area to be converted. The probability of polygon creation at any location on the landscape then becomes:

$$P_h = \frac{N_h}{A_h}$$

where  $N_h$  is the number of polygons of habitat type  $h$  to be converted.

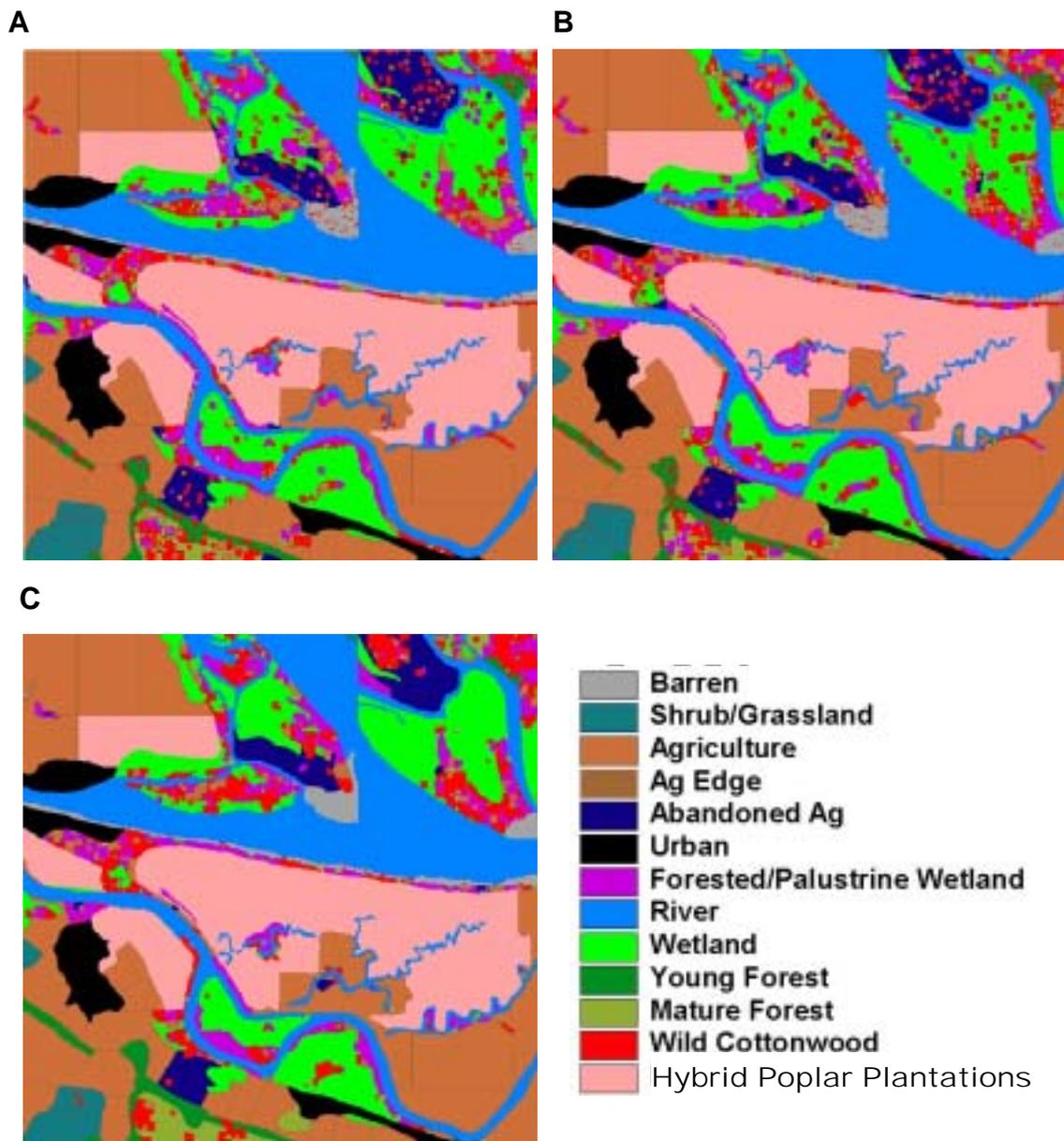
One further complication is that probability of polygon creation depends on spatial context of the nucleation point. For example, new poplar polygons are more likely to be aggregated to existing poplar polygons than to be created in isolation. Similarly, poplar mortality is more likely to occur at the edges of a stand rather than in the interior.

Therefore, we forced a proportion of the new polygons to aggregate to existing poplar stands each year.



**Figure 2.9** Distributions of sizes of poplar patches that appeared (establishment, **A** and **B**) and disappeared (mortality, **C** and **D**) under pre-dam (1961-1973, **A** and **C**) and post-dam (1983-1991, **B** and **D**) disturbance regimes.

Each year we first count all cells of each habitat type on the landscape, enumerating ‘interior’ and ‘edge’ cells (poplar stand edges, and nonpoplar cells that abut poplar edges). We then calculate the total area to be converted to and from poplar for each habitat type and sample the number of polygons required to convert that area. Then we divide the polygons between ‘interior’ and ‘edge’ conversions based on a user-defined parameter that controls the degree of aggregation (Figure 2.10A-C), and calculate separate conversion probabilities for each. Finally, we generate a random number for each cell on the landscape and determine if poplar mortality or establishment will occur



**Figure 2.10** Effects of aggregation factor on sizes of established cottonwood patches following 50 years of simulation. The aggregation factor is the proportion of poplar polygons that are established and removed adjacent to a an existing wild poplar polygon. Increasing this value enhances amalgamation of new poplar patches with existing patches. **A.** Aggregation factor=0. **B.** Aggregation factor = 0.3. **C.** Aggregation factor = 0.7 (the default condition for subsequent model runs).

at that point. We then convert all cells of the same habitat type up to the predetermined polygon size, or until the entire existing polygon has been converted.

Agricultural fields represent a special case because they are consistently subject to anthropogenic influences. However, the edges of agricultural fields are often subject to colonization by poplar, especially in cases where the fields are adjacent to drainage ditches and roads. We therefore allowed colonization of field edges with a customizable probability and maximum patch size. Also, poplars may colonize fields containing extensively managed perennial species such as tree plantations in some areas. We therefore simulated managed lands that were not subjected to annual tilling or grazing and were thus susceptible to poplar establishment. Establishment within such fields was limited to a single cell (100m<sup>2</sup>) with establishment and mortality rates set by the user. Mortality in agricultural fields is also controlled by herbicide application, and modulated by genotype-specific herbicide tolerance (see Herbicide Tolerance scenario, Chapter 4).

Finally, large-scale abandonment of agricultural land has been common in this study area, and some of this land ultimately succeeds to poplar stands. For example, the Lewis and Clark National Wildlife refuge was established in the study area in 1971, and diked agricultural land comprised large expanses of this refuge (Allen 1999). The dikes were eventually breached, and poplar stands developed by 1991. We therefore calculated the rate of conversion to poplar of known abandoned agricultural land, and applied this rate to fields that were abandoned at set intervals during model runs. Similarly, conversion of poplar stands to agriculture has been relatively common, and we included this transition as well.

#### *Assumptions and Limitations*

Some of the advantages to our approach are computational efficiency, ease of parameterization, and ease of customization. Chronosequences of air photos are widely available, and calculation of conversion rates is relatively straightforward using a GIS platform such as Arc/Info, or simple computer programs to compare binary files (available from authors). The disturbance regime is easily altered for this model because rates of establishment and mortality, degree of aggregation, and minimum disturbance rates are all specified in text files that are input at model initiation.

This simplicity does entail some major assumptions and limitations, however. Key assumptions include:

1. *Differences in delineated habitat types between data layers represent actual landscape changes.* In fact, there is a tremendous amount of interpretation inherent in habitat designations, and a large number of people were involved in delineating and digitizing the data in the original project, so a certain amount of the apparent transitions were likely artifacts of the delineation and digitization process. However, the data were synthesized, corrected, and subjected to extensive ground-truthing (Allen 1999), and usage of merged habitat types should further alleviate these problems. Also, in calculating transition rates we only used polygons of 0.1 ha or greater, which should reduce misclassification errors.
2. *Relationships between poplar occurrence and habitat type in 1991 are representative of relationships at earlier time points.* Hydrologic regimes have presumably changed substantially over the course of the chronosequence, and it is reasonable to expect that the poplar component might have changed in some of the habitat types (*e.g.*, the representation of poplar in forested wetlands might have increased relative to willow due to lower moisture conditions, while presence in broadleaf forest might have declined).
3. *Prevailing disturbance regimes from the past can be projected into the future.* Once again, changes in the hydrology and management of this and surrounding areas could drastically alter the disturbance regimes into the future, and such effects are difficult to quantify and impossible to predict. Our approach is to use the past conditions as a baseline to begin simulations, and to assess the effects of vastly different disturbance regimes on model outcomes.
4. *Areal probability can be converted to probability of polygon formation.* The disconnect between the simulation unit (the cell, a unit of area), and the disturbance unit (a polygon) is problematic. One consequence is that our method is insensitive to low-probability conversion events, particularly for small landscapes where the maximum number of cells converting will be less than one, and probability of conversion will be zero because of rounding error. To

compensate for this we have incorporated a minimum probability of conversion for all habitat types that can transition to poplar.

5. *Establishment and mortality of poplar are the major transitions driving poplar succession.* There may be intermediate steps in the conversion of the landscape to and from poplar stands. It is reasonable to assume, for example, that poplar might sometimes succeed to ‘mature forest’ containing other species, which may in turn convert to wetland or barren land before ultimately reverting back to poplar. Similarly, barren land might first convert to wetland, followed by sedimentation and/or disturbance and conversion to poplar. However, poplar is primarily a pioneer species that can occupy sites for very long periods, yet it is susceptible to catastrophic disturbance due to its often precarious position in the flood plain (Braatne, Rood, and Heilman 1996). Therefore, it is reasonable to assume that transitions can occur almost directly to and from poplar much of the time. Furthermore, we lacked adequate data on transitions among all possible habitat types, and highly erroneous estimates for some of these parameters could have skewed the model in unpredictable ways. Consequently, our landscape appears more homogeneous than might otherwise be expected following 100 years of simulation, particularly with regard to the high component of wetland (which has a low transition rate directly to poplar), and forested wetland (which has a high transition rate from poplar) (Figure 2.10C). Therefore, a logical and desirable extension of this model would be the incorporation of a more realistic disturbance regime that incorporates these intermediate transitions.
6. *Model outcomes are relatively insensitive to polygon shape.* For the sake of computational economy, we used a simple sequential search algorithm for the formation of new polygons. This algorithm results in straight-edged polygons when the edge of the new polygon is interior to an existing polygon, but new polygons assume a more natural shape when they encounter the edge of an existing polygon (Figure 2.10C). This algorithm causes increasingly angular and square shapes as the simulation progresses, rather than the more linear and curved polygons of natural systems. However, some more natural shapes persist as well.

## **Production of Pollen, Seeds, and Vegetative Propagules**

### *Primary Data*

The primary purpose of this model is to explore the process of gene flow from transgenic poplar plantations. The basic simulation unit is a 100 m<sup>2</sup> cell, which approximately represents one fully grown tree. However, the absolute number and size of trees is not important in this model, because it operates primarily on relative proportions of transgenic and conventional genotypes. This is fortunate, because data on growth and productivity of wild poplar stands are largely lacking. However, it was important to derive a relationship between basal area and age, because increases in size and fecundity should decline with time. Therefore, we used the limited, anecdotal data available to us on ‘culmination’ of annual increment (DeBell 1990) in wild populations to derive a relationship with basal area (Figure 2.5).

We related seed, pollen, and vegetative propagule production directly to basal area, because basal area is correlated with crown size, which in turn largely determines fecundity (Greene and Johnson 1999). We also assumed that larger crowns have a greater chance of producing the broken limbs and secondary shoots that comprise dispersed vegetative propagules. Seed and pollen production are further modulated by sex ratio and genotype-specific fecundity, which allows for simulation of transgenic sterility.

There is substantial concern about possible instability of transgenic traits, and the prospect that genetically engineered sterility could become ineffective under the relatively long rotations and varied environments encountered in tree plantations. We therefore explored the effects of instability on genetically engineered sterility by allowing genotype-specific fecundity to vary stochastically through time.

Seed and pollen production occur after trees reach maturation age, which typically occurs around 10 or 15 years for wild trees, and around 5 years for plantation trees in the study area (Stanton and Villar 1996).

### *Implementation in Model*

Relative pollen production is calculated for each genotype within each sexually mature cohort of trees in each poplar cell. Representation of pollen and seed is entirely relative, because the most important quantity is the ratio of transgenic to conventional genotypes in the propagule pools. Therefore, pollination units,  $P_g$ , are unitless, and calculated as:

$$P_g = Ba_{ga} f_{ga} Sr_g$$

where  $Ba_{ga}$  is basal area (already defined),  $f_{ga}$  is relative fecundity of genotype  $g$  at age  $a$ , and  $Sr_g$  is sex ratio of genotype  $g$  within the cohort. Relative fecundity can vary annually based on a user-defined standard deviation. In addition, transgenics with reduced fecundity can have fecundity partially restored according to a user-defined probability.

Vegetative propagule production is calculated as:

$$V_g = Ba_{ga} R$$

where  $R$  is a random number between 0 and 1.

Production of transgenic seeds (genotype 1) by all plantation and wild trees is calculated as:

$$S_{1a} = (f_{1a} Ba_{1a} (1 - Sr_1) + f_{0a} Ba_{0a} (1 - Sr_0)) K_1$$

where  $K_l$  is the proportion of compatible transgenic pollen arriving at the stigma.  $K$  depends on pollen input from local sources, background sources, and phenological compatibility (described in pollination section). This equation incorporates both pollination of conventional trees by transgenic pollen as well as seed production by transgenic females.

Similarly, conventional (genotype 0) seed production is calculated as:

$$S_{0a} = (f_{0a} Ba_{0a} (1 - Sr_0)) K_0.$$

### *Assumptions and Limitations*

1. *Age-specific fecundity does not differ substantially between plantation and wild trees.* It is reasonable to expect that growth of plantation trees will exceed that of

wild trees, both because of superior growth conditions and because of hybrid vigor (Stettler et al ). However, hybrids typically have lower fertility than wild trees on a per-catkin basis (Chapter 3), and it is unclear whether increased catkin production at early ages can compensate. In any case, the model allows later maturation of wild trees than plantation-grown trees (10 yr and 5 yr respectively), which reflects some of the differences in plantation versus wild conditions (Schreiner 1971).

2. *Pollen, seed, and vegetative propagule production are directly proportional to basal area of cohorts.* Seed and pollen production have been shown to be linearly associated with basal area for a wide variety of tree species, though there can be deviations from this relationship at basal areas above 0.4 m<sup>2</sup> (Greene and Johnson 1994).
3. *Productivity does not increase after 200 years.* Data on age structure of natural poplar stands are lacking, but it is reasonable to assume that senescence is common by 200 years. In any case, given the dynamism of this landscape and the typical duration of simulation runs, few trees older than 200 years will typically be present on the modeled landscape.

## **Pollination**

### *Primary Data*

#### Pollen Dispersal

Pollen dispersal is potentially a key mechanism for dispersing genes from transgenic plantations. Accordingly, we expended substantial effort in characterizing factors associated with pollination success (unpublished data). In particular, we derived a relationship between pollination success and distance using paternity analysis. These methods are described in detail elsewhere (Chapter 3). Briefly, we collected seeds from a number of female trees growing within wild poplar stands at three divergent sites in Oregon. We extracted DNA from the seeds, mothers, and potential fathers within a local sampling area, and used paternity exclusion analysis and the ‘most likely’ method to

assign seeds to fathers. Seeds not assignable to fathers within the local area were considered to result from ‘gene flow’ from outside of the sampled area (Table 2.5). We then plotted distance versus pollination success for all seedlings for which parentage could be attributed (local pollination) (Figure 2.11), and used nonlinear regression to fit the data to an exponential distribution (Figure 2.12).

**Table 2.5** Results from paternity analyses at 3 sites.

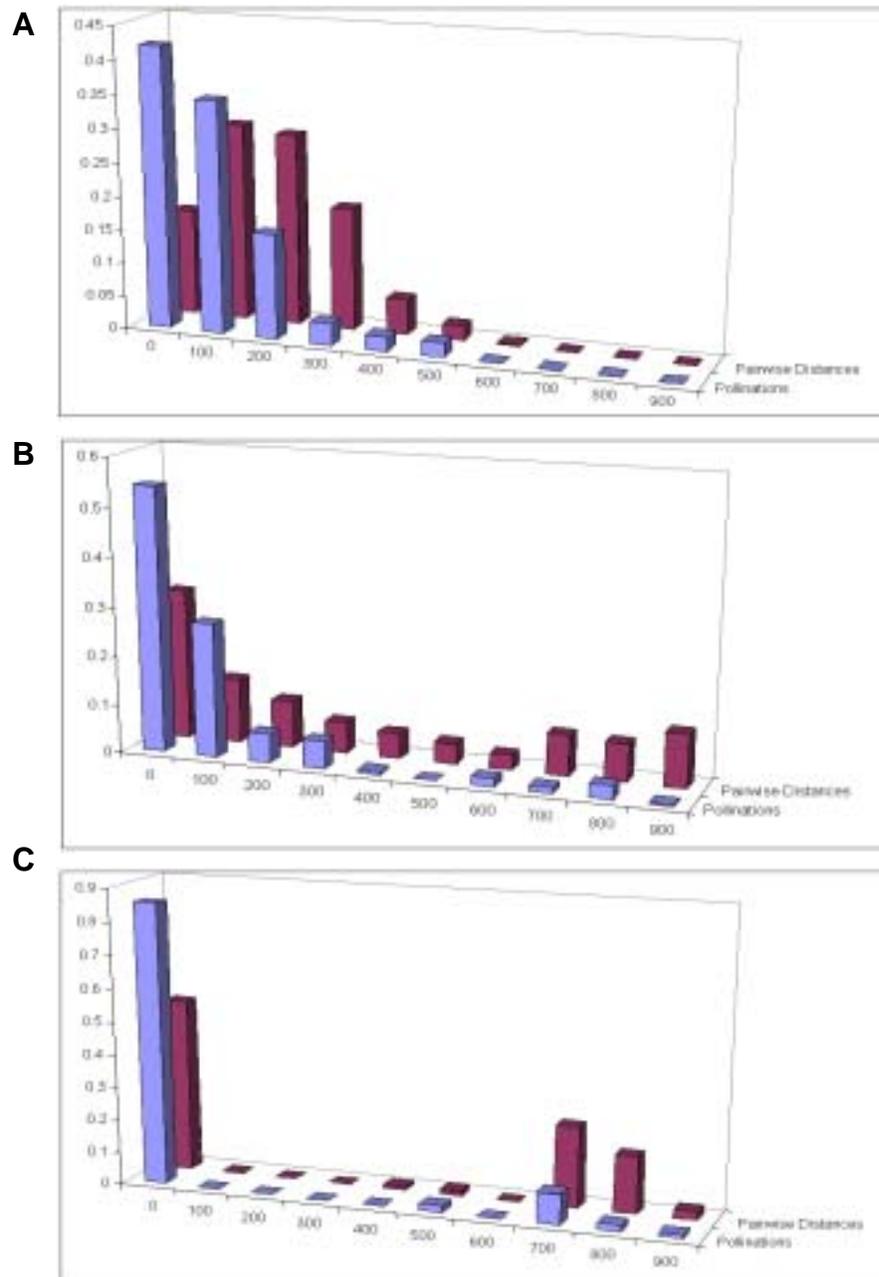
Site	Radius of Sampled Area	Mother Trees Sampled	Potential Fathers	Progeny Sampled	Paternity Assigned to Single Male	Multiple Paternity	Gene Flow (%)
Willamette	0.25	5	221	239	103	27	42
Luckiamute	1	5	57	423	45	4	75
Vinson	10	28	54	849	355	29	58

### Phenology

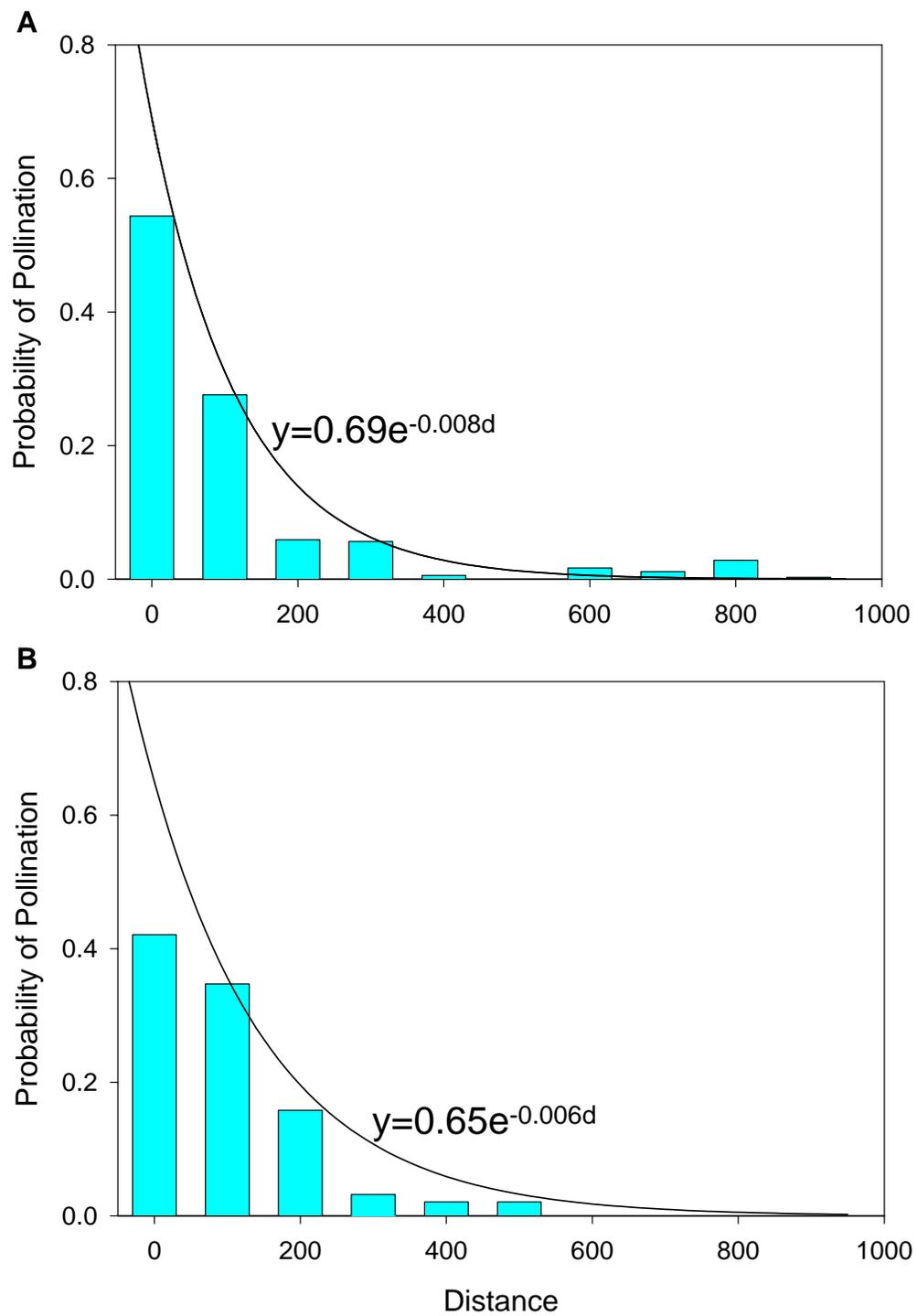
We estimated phenological compatibility among trees by calculating the number of days of overlap in flowering time for all male and female trees within stands. We estimated flowering phenology for individual genets by repeated observations of flowers during the period of anthesis at three sites (Chapter 3). We devised a scoring system that could be implemented from the ground with binoculars, and which reflects pollen shedding (for males) and receptivity (for females) (Table 2.6). We recorded phenology observations for up to three years, and determined the mean duration of flowering. We then extrapolated the start dates for receptivity as follows:

$$S = d_{pe} - D \frac{S_e - S_r}{S_a - S_r}$$

where  $d_{pe}$  is the earliest day on which receptive stigmas were observed,  $D$  is the average duration of receptivity or pollen shedding,  $S_e$  is the earliest receptive or shedding stage observed,  $S_r$  is the stage at which receptivity or shedding begins, and  $S_a$  is the stage at which receptivity or shedding is complete.



**Figure 2.11** Relationship between local pollination success and distance. Pollination success (front row) is the proportion of observed pollinations that occurred within each distance class. Observed pollinations represent between 26% and 57% of all pollination, with the rest coming from outside the sampled population (gene flow, Table 2.5). All pairwise distances between males and females (back row) are also plotted for reference: this represents a null expectation for pollination success if distance is unimportant. **A.** Willamette. **B.** Vinson. **C.** Luckiamute.



**Figure 2.12** Fitting of negative exponential equation to pollination data derived from paternity analyses. Curves were fit using nonlinear regression. **A.** Vinson site. **B.** Willamette site

The end date of flowering was calculated as:

$$E = S + D.$$

We then calculated the least square mean of flowering date among all years, and calculated the pairwise overlap in flowering among all individuals in the population, with negative numbers representing the number of days separating individuals that did not overlap (Figure 2.13).

**Table 2.6** Classes used in phenology measurements. Bold classes indicate at least some pollination is occurring.

#### **Males**

- 1-- Catkins not emerging
- 2-- Catkins emerging, but compact
- 3-- <50% of catkins opening
- 4-- >50% of catkins opening
- 5-- <50% of catkins shedding pollen**
- 6-- >50% of catkins shedding pollen**
- 7-- 50-90% of catkins abscised; vegative bud break
- 8-- Post-pollination; all catkins abscised

#### **Females**

- 1-- Catkins not emerging
- 2-- Catkins emerging, but compact
- 3-- <50% of bracts open**
- 4-- >50% of bracts open**
- 5-- <50% of capsules distinct**
- 6-- >50% of capsules distinct**
- 7-- stigmata brown or abscised; vegetative bud-break
- 8-- pubescent sutures
- 9-- seeds shedding

#### *Implementation in Model*

##### Distance

For the purposes of the simulation we divided pollen dispersal into two processes: local pollination, which is sensitive to the effects of distance between potential mates, and background pollination, for which distance is not a determinant (Clark et al 1998; Higgins and Richardson 1999). We defined the size of local pollination ‘neighborhood’

as the distance at which pollination success reaches background levels (*i.e.*, the point at which distance between mates is not a significant determinant of reproductive success). This value was between 400 and 500 m for both the Willamette site and eastern Oregon (Figure 2.10). We dispersed pollen to each poplar cell containing females by searching a neighborhood of 440 m radius, discounting pollen input by distance between the source and target cells as follows:

$$D_p = \beta e^{-\chi d}$$

where  $D_p$  is the scaling factor for distance between source and target cells,  $d$  is distance between mates, and  $\beta$  and  $\chi$  are means of parameters fit by nonlinear regression to two observed distributions of pollen (Figure 2.12). This resulted in a very large number of searches for our full landscape:  $1.5 \times 10^9$  cells with 200,000 female poplar cells. In an effort to minimize the number of calculations, we created links among all poplar cells on the landscape and devised a search algorithm to minimize the number of cells processed. Nevertheless, the pollen dispersal algorithm accounts for the vast majority of processing time for this model.

### Wind

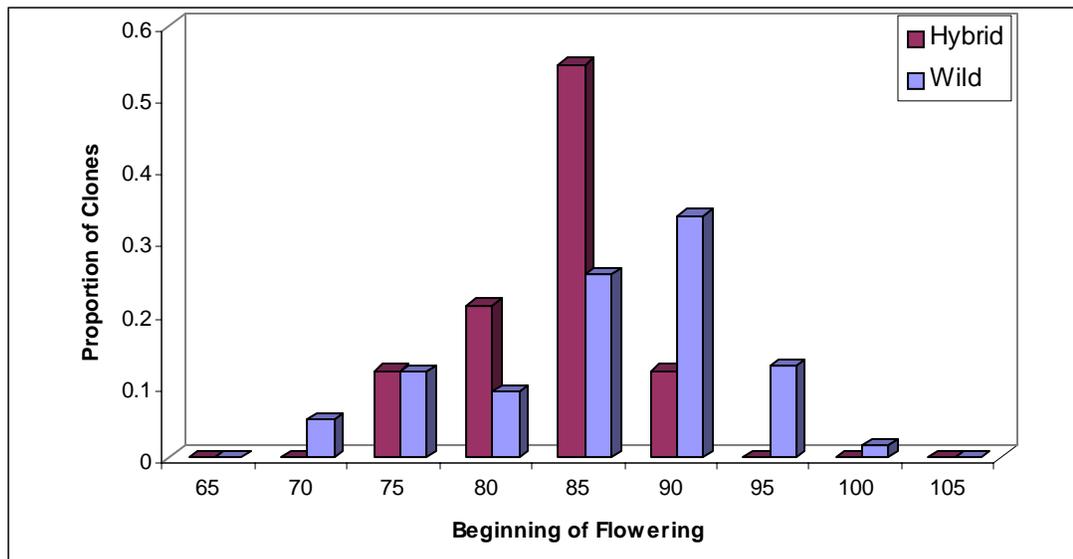
We simulated the effects of wind speed and direction by using the scalar product between the prevailing wind vector and the vector between the source and target cells, scaled by a factor representing wind speed:

$$W = \delta - \varepsilon \cos \theta,$$

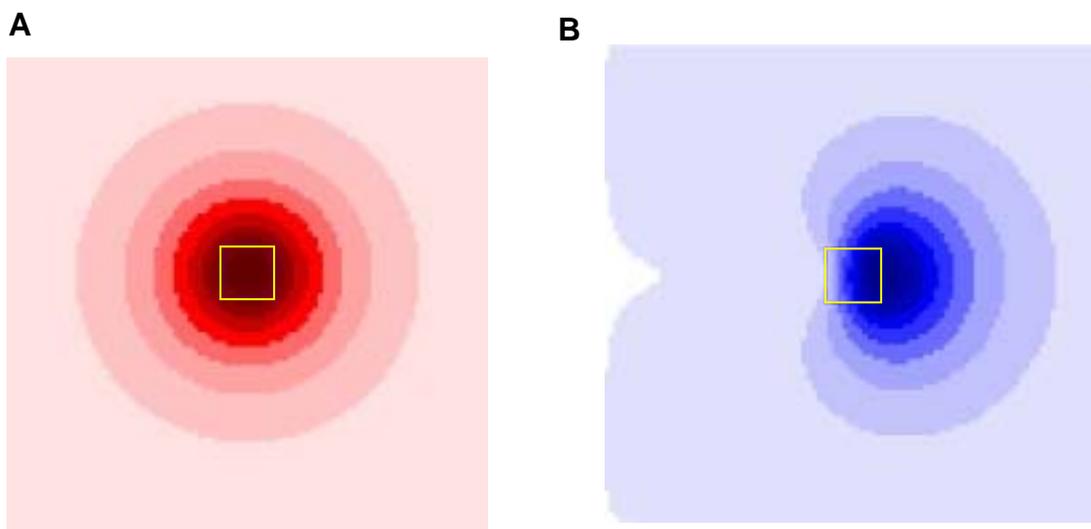
where  $\delta$  and  $\varepsilon$  are parameters that vary between 0 and 1, and  $\theta$  is the angle between the prevailing wind vector and the vector from source to target cells (Figure 2.14).

### Phenology

Our phenology data indicated that the clones currently grown in plantations in the Pacific Northwest flowered earlier on average than sympatric native trees, but there was still extensive overlap in flowering (Figure 2.13). Others have reported substantial discontinuity between native and plantation-grown poplars in phenology of flowering and seed dispersal, particularly in more severe climates (U.S.Environmental Protection Agency 1999); Barbara J. Thomas, University of Alberta, personal communication).



**Figure 2.13** Frequency distribution of flowering dates of male and female *P. trichocarpa* and hybrid trees at the Willamette River site, Oregon. Start dates of flowering were extrapolated from the least square means of flowering observations for 1996, 1997, and/or 1998 for 426 *P. trichocarpa* genotypes and 33 hybrid clones (mostly *P. trichocarpa* x *P. deltoides*).



**Figure 2.14** Pollen dispersal and the influence of wind. **A.** No wind. **B.** West wind, with scaling factor (wind speed) set to 0.5 (default). Shading is proportional to the probability of pollination. Landscapes are 800 m on a side, and central yellow squares represent a 100m x 100m male plantation.

Therefore, we have devised a flexible method for simulating the effects of phenological discontinuities on pollination. The first step is to define the number of phenology classes (*e.g.*, early, middle, late) supported by data. The number of classes, together with an incompatibility factor, determine the degree of phenological compatibility between trees of different classes:

$$H = \frac{(N_p - \phi) - |p_1 - p_2|}{N_p - \phi}$$

where  $N_p$  is the number of phenology classes,  $p_i$  is the phenology score (range 1 to  $N_p$ ) of clone  $i$ , and  $\phi$  is an incompatibility parameter, set for the entire population between 0 and  $N_p-1$ . Values of  $H$  of 0 or less indicate complete incompatibility. This technique allows simulation of a broad gradation of intercompatibility (Figure 2.15A). Furthermore, plantation and wild trees can be assigned different phenology distributions, allowing simulation of phenological discontinuities. For our simulations we examined three distributions of phenology each for plantation and wild trees: observed distributions, uniform distributions (equal number in each class) and nonoverlapping distributions (all plantation trees in early class, all wild trees in late class(es)). For each set of distributions, we tested cases with two and three phenology classes, and  $\Phi = 1$  and 2. This resulted in a range spanning complete compatibility between plantations and wild trees, and complete incompatibility due to disjunct flowering (Figure 2.15B).

### Pollination

Pollination success of each genotype is a function of the relative quantity of compatible pollen arriving in the target cell. Alternatively, this can be viewed as a function of the cumulative probability of pollination by that genotype, summed over all cells on the landscape. This can be a function of distance, phenological overlap, and/or direction. Pollen input (or probability of pollination) of each genotype  $g$  from each cell  $i$  in the neighborhood of the target cell is calculated as

$$P_{gi} = P_g D_p W H.$$

The proportion of seeds of each genotype is determined from the proportional representation in the pollen arriving in the cell (see seed production equation above). A

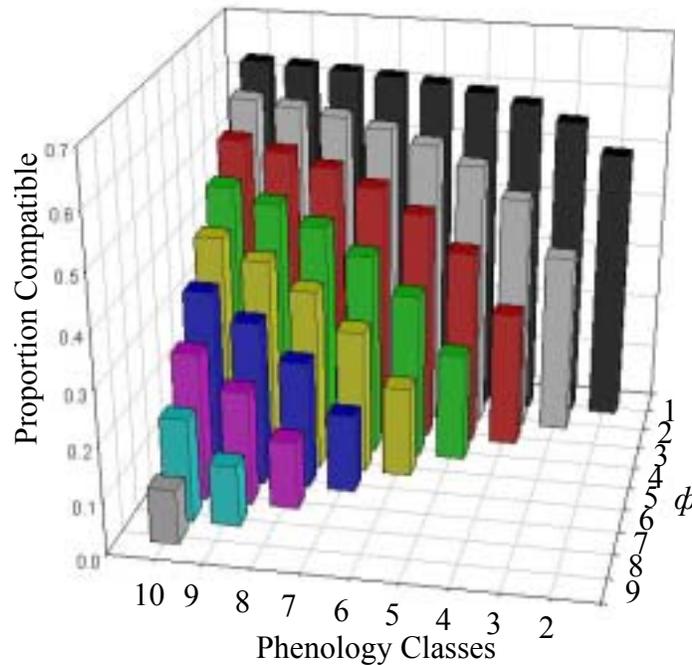
certain proportion of the pollinations ( $\gamma$ ) derive from local trees in the neighborhood, and the remainder from distant trees (the ‘background pollen cloud’, estimated as 0.5 from our gene flow studies). Therefore, for  $i$  cells in the neighborhood, and a landscape with  $j$  poplar cells, the proportion of compatible pollen of genotype  $g$  arriving at the cell is:

$$K_g = \gamma \frac{\sum_i P_{gi}}{\sum_g \sum_i P_{gi}} + (1 - \gamma) \frac{\sum_j P_{gj}}{\sum_g \sum_j P_{gj}}.$$

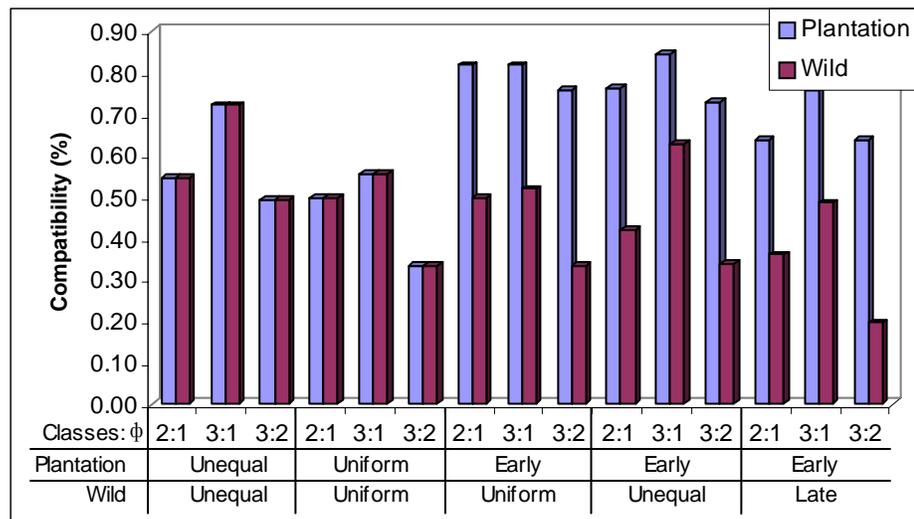
### *Assumptions and Limitations*

1. *Pollen availability does not limit seed production.* We assume that pollen limitation is not a significant factor limiting seed production in this landscape, and that there is adequate pollen in the background pollen cloud to fertilize all available ovules. This assumption will assuredly be violated to some degree: pollen limitation is commonly observed in trees and other plants, as determined by pollen addition experiments (Burd 1994; DiFazio et al 1998). However, seed set was uniformly high at these study sites, and poplars are characterized by extremely high fecundity and vagility of pollen and seed. Also, the model can be made sensitive to isolation from male trees by setting  $\gamma$  (local pollination) to 1: seeds will then not be produced in the absence of local pollen production.
2. *Hybrid and wild pollen are equally fertile.* This assumption is known to be incorrect, as demonstrated in numerous greenhouse crossing experiments (e.g., Chapter 3). However, the model does allow for simulation of decreased fertility of transgenic trees, which alleviates the problem. Furthermore, there is a tremendous amount of variation in fertility of crosses involving hybrid trees as well as crosses among wild trees (Stanton and Villar 1996), so assuming equal fertility is a conservative approach to assessing safety of transgenic trees.
3. *Transgenes are dominant, and present in multiple, unlinked copies.* We do not track segregation of the transgene: all progeny of transgenic trees inherit the transgenic phenotype. In reality, commercial transgenic trees will likely have a single copy of the transgene, which will segregate in a Mendelian fashion

A



B



**Figure 2.15** Effects of flowering phenology on compatibility between trees. **A.** Effect of number of phenology classes and compatibility factor on relative interclone compatibility. Interclone compatibility is expressed relative to maximum intercompatibility between clones (one phenology class), assuming equal numbers of clones in each class. **B.** Interclone compatibility with two or three phenology classes, and a compatibility factor of 1 or 2, and different distributions of trees among phenology classes for plantation and wild trees. Unequal, trees distributed according to observed distributions (Figure 2.12). Random, equal numbers of trees in each class. Early, all trees in earliest phenology class. Late, no trees in earliest phenology class.

in the progeny. This is equivalent to STEVE simulations with transgenic fertility of 50%.

## **Dispersal of Propagules**

### *Primary Data*

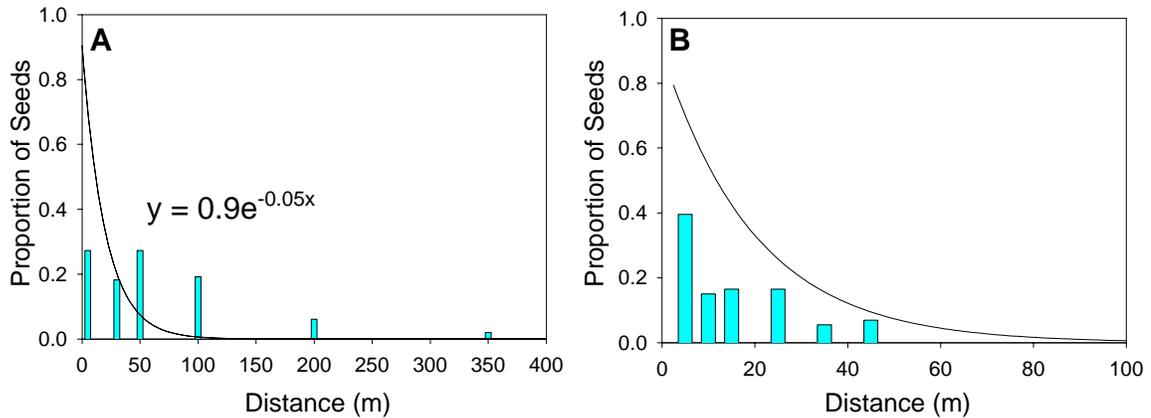
#### Seed Dispersal

We estimated seed dispersal both by direct measurements of seed movement, and by using maternity analysis on seedlings and seeds captured in the vicinity of plantations. We performed direct measurements by setting seed traps at various distances from isolated wild poplar trees, and from the edge of isolated blocks of hybrid plantations. These traps consisted of 0.25 m<sup>2</sup> wire mesh coated with “Stikem Special” adhesive (Seabright Laboratories, Emeryville, CA), and mounted on 1 m wooden posts. We counted and removed seeds once every 7 days for two weeks (Figure 2.16).

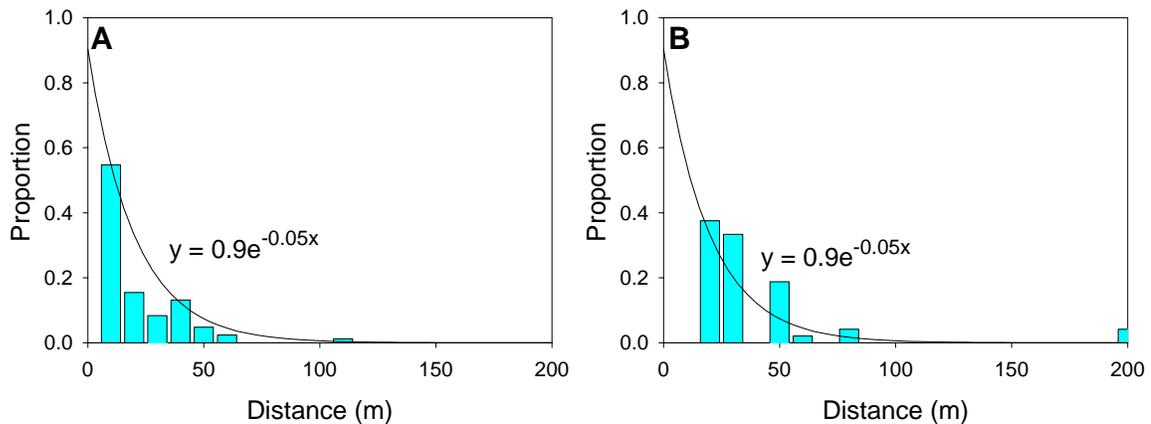
We also collected seeds in the vicinity of plantations using mesh bags suspended on a wooden frame. We emptied traps twice weekly, germinated seeds, and identified hybrids based on leaf morphology, and Random Amplified Polymorphic DNA (Welsh and McClelland 1990). We also used microsatellites (Morgante and Olivieri 1993) to identify specific mother trees, and calculated dispersal distances (Chapter 3; Figure 2.17).

#### Vegetative Dispersal

We had little direct data on vegetative dispersal for this study, so we chose to rely on a retrospective assessment of clone size as an integrated index of successful vegetative dispersal distances. We estimated genetic distances among ramets by the total difference in estimated microsatellite allele size for 10 or more loci. We analyzed these data using the Unweighted Pair Group Method with Arithmetic mean (UPGMA (Sneath and Sokal 1973) to identify putative clones, accounting for somatic mutations and scoring errors within a clone (Tuskan *et al.* 1993). We confirmed these identifications with field observations of phenology, sex, and morphology. We then calculated the minimum and mean distance between individual ramets and all other ramets of the clone (Figure 2.18).



**Figure 2.16** Capture of seeds by traps. **A.** Traps situated east of an isolated female tree. **B.** Traps situated east of an isolated 10 ha block of a female hybrid poplar clone.



**Figure 2.17** Seed movement determined by maternity analysis. Traps were placed near plantations, and maternity was assigned to closest genetically compatible tree (based on microsatellite profiles). Line is the negative exponential equation used in the STEVE model for seed movement. **A.** Willamette. **B.** Columbia.

### *Implementation in Model*

Seeds are dispersed explicitly only in the direct vicinity of establishment sites. We fit a negative exponential curve to the frequency distribution of dispersal distances (Figures 2.17 and 2.18), and seed and vegetative dispersal are implemented much like pollen dispersal, except the default neighborhood is 220 m rather than 440 m. Local seed dispersal is also subject to the influence of wind, so that seed input from each source cell,  $i$ , is:

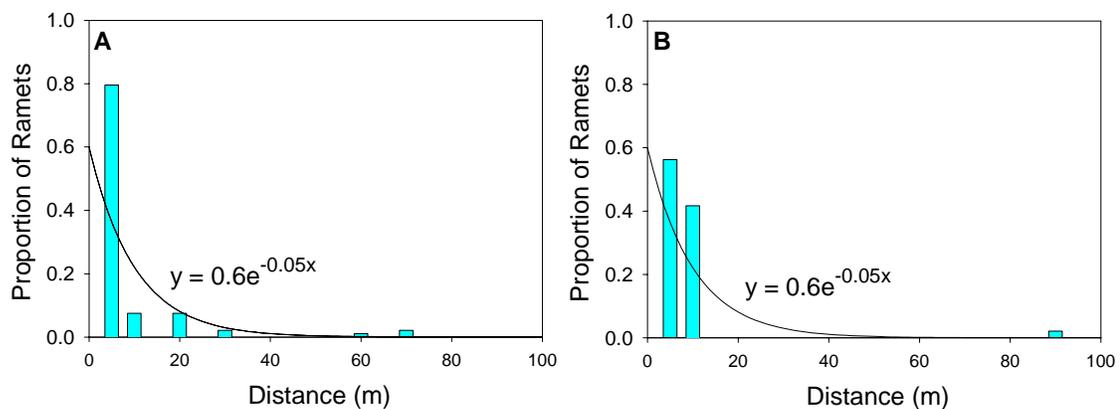
$$S_{gi} = S_g D_s W$$

where  $S_g$  is seed production for genotype  $g$  (defined above),  $D_s$  is the relationship between seed dispersal and distance, and  $W$  is the effect of wind (same as for pollen). In addition, total production of seeds of each genotype is tallied on the landscape to allow for a ‘background’ seed cloud.

Input of vegetative propagules from each source cell,  $i$ , only depends on distance:

$$V_{gi} = V_g D_v.$$

where  $D_v$  is the relationship between vegetative propagule dispersal and distance.



**Figure 2.18** Minimum pairwise distances between ramets of clones. Clone delineation was based on microsatellite genotypes and clustering by UPGMA. We infer that these distributions are representative of effective clone dispersal distances, and the lines represent a negative exponential equation used in the STEVE model to depict vegetative dispersal. **A.** Willamette. **B.** Columbia.

#### *Assumptions and Limitations*

1. *Vegetative dispersal is primarily local.* This assumption is supported by the limited data available to us, insofar as the maximum distance among ramets observed was 98 m. Also, a survey of the literature on clonal reproduction in poplar revealed maximum observed inter-ramet distances of 77 m (Table 2.7), which is well within the neighborhood in which we perform explicit dispersal. However, these surveys were all biased toward discovering only local dispersal because they necessarily took place on a local scale. It is likely that episodically effective long-distance dispersal of vegetative propagules occurs at a low rate. To

some degree, this phenomenon is captured in the model by background seed establishment, though this is not the case when sterile plantations are simulated.

2. *Current clone structure reflects vegetative dispersal distance.* Individual clones can be quite old, and the structure is sure to change through time due to mortality of ramets (Cook 1985). Therefore, it can be quite misleading to use inter-ramet distances to estimate dispersal distances. However, current inter-ramet distances will likely overestimate historical dispersal of individual propagules (assuming intervening ramets have died), so this is a conservative assumption regarding potential spread of transgenic trees from plantations.

## **Establishment**

### *Primary Data*

#### Density and Extent

Data on poplar establishment come primarily from experimental plots we established in the vicinity of poplar plantations at two sites, Columbia and Willamette (DiFazio *et al.* 1999). We cleared 1 m<sup>2</sup> plots in the vicinity of competing vegetation and monitored input of poplar seeds, and emergence and growth of seedlings over two years. We repeated the experiment for two years at the Columbia site and three years at the Willamette site. However, despite weekly supplemental watering, we only observed substantial establishment in the first year of the study, 1996 (Figure 2.19), which was the year of a large flood and elevated water tables. Such episodic establishment is to be expected for this species (Auble and Scott 1998; Braatne, Rood, and Heilman 1996; Bradley and Smith 1986; Scott *et al.* 1997; Stromberg 1997).

#### Propagule Type

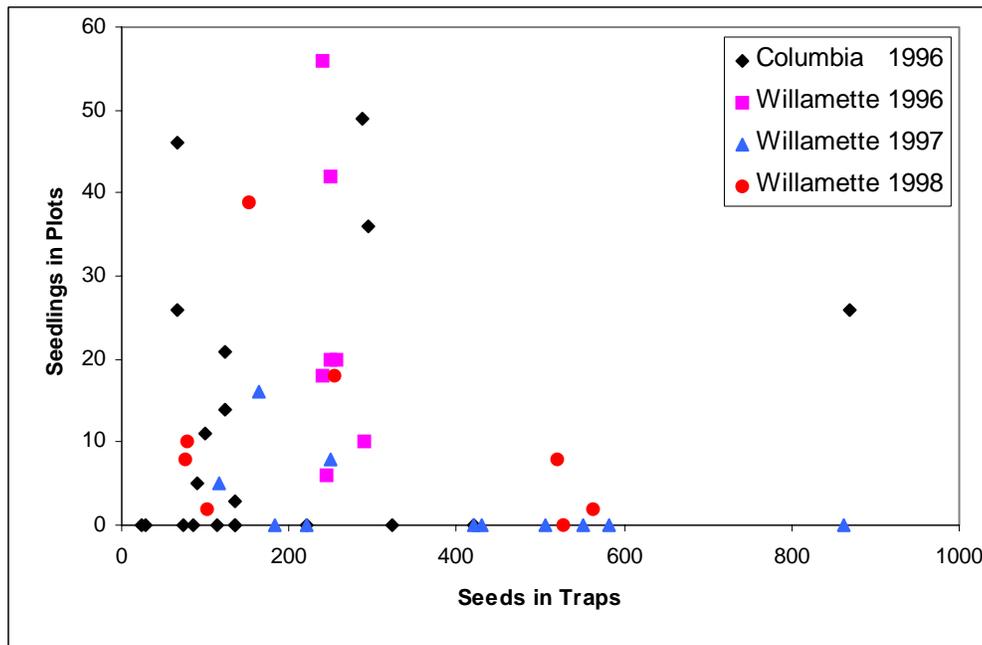
Analogous to pollination, establishment of each genotype is determined primarily by proportional representation in propagules dispersed to the cell. However, the situation is more complicated for establishment because there are several propagule types: locally

**Table 2.7** Frequency and extent of cottonwood clones in the wild for a variety of studies.

Site	Ramets	Genets	Clonality (%) <sup>1</sup>	Distance Between Ramets (m)	Long Distance (%) <sup>2</sup>	Source
Lower Nisqually	62	61	2	-	-	McKay 1996
Lower Cowlitz	35	29	17	-	-	McKay 1996
Upper Nisqually	55	46	16	-	-	McKay 1996
Upper Cowlitz	52	43	17	0-77	-	McKay 1996
Lethbridge	194	29	85	1.2-9.2	0	Gom and Rood 1999
Yakima 1(lower)	51	46	10	<10	0	Reed 1995
Yakima 2(lower)	48	48	0	<10	0	Reed 1995
Yakima 3(upper)	18	32	36	<10	0	Reed 1995
Yakima 4(upper)	25	27	48	<10	0	Reed 1995
Beaugency (nigra)	118	114	3	<5	0	Legionnet et. al. 1997
Oldman River	57	43	75	-	~1	Rood et al. 1994
Fraser R., Similkameen R.	Many	?	~5%	-	<1	Galloway and Worrall 1979
Davidson	104	57	45.2	-	-	Unpublished data
Willamette	287	221	31.4	0.6-76	6.6	Present study
Clatskanie	94	57	45.7	2.8-98	20	Present study
River Ranch	54	45	17	-	-	Present study

<sup>1</sup>Clonality is the percentage of ramets in the stand that had at least one other identical ramet in the stand.

<sup>2</sup>Potential long-distance dispersal. Includes ramets separated by 10 m or more (Nisqually, Cowlitz, Willamette, Clatskanie), or ramets resulting from small propagules (Oldman, Fraser/Similkameen), which presumably could have resulted from long-distance dispersal.



**Figure 2.19** Establishment of seedlings in artificially disturbed plots near plantations. Establishment versus seed input for 4 different experiments. Experiments were performed at two sites in 1996 and only at the Willamette site in 1997 and 1998. Seeds were collected in 0.25 m<sup>2</sup> traps adjacent to 1 m<sup>2</sup> plots. Seedlings were counted in August of the year of establishment.

produced seeds, background seeds, and vegetative propagules. Unfortunately, data on the relative success of these different propagules are generally lacking. However, we were able to derive estimates of vegetative versus seedling establishment based on inferences from existing clone structure, and we can infer likely ranges of long-distance seed dispersal based on propagule characteristics and expectations for physical dispersal.

We estimated rates of vegetative versus seedling establishment at 4 sites as

$$V = 1 - \frac{G}{R}$$

where  $G$  is the number of genets and  $R$  is the total number of ramets.

We also derived similar estimates for a variety of published studies on *P. trichocarpa* and the ecologically similar species *P. nigra* (Table 2.7).

From the perspective of this simulation model, vegetative establishment directly adjacent to the parent tree is treated as vegetative growth, because this occurs within a cell, which is the unit of simulation. Therefore, we differentiated long-distance (> 10 m) from local vegetative dispersal in calculating rates of vegetative establishment (Table 2.7).

There have been few direct studies of long-distance seed dispersal in trees (Clark, Fastie, Hurtt, Jackson, Johnson, King, Lewis, Lynch, Pacala, Prentice, Schupp, Webb III, and Wyckoff 1998; Higgins and Richardson 1999), and none in poplar. Furthermore, the genetic data generated for this study were intended primarily for studying pollen dispersal and for differentiating plantation from wild seed sources, and they are largely inadequate for tracking long-distance seed flow (Chapter 3). Poplar seeds are tiny (~0.4 mg dry weight, personal observation) with plumed appendages that facilitate dispersal by both wind and water (Braatne, Rood, and Heilman 1996). However, our observations of local seed dispersal (Figures 2.16 and 2.17) indicated that seeds do not move as far as pollen (Figure 2.11), and we can therefore expect that background seed input will be less than background pollen input.

### *Implementation in Model*

Establishment of each genotype in a neighborhood of  $i$  cells on a landscape with  $j$  cells is proportional to its representation in each of the propagule pools, as follows:

$$L_g = RL_{\max} \left( \kappa \frac{\sum_i S_{gi}}{\sum_g \sum_i S_{gi}} + \lambda \frac{\sum_j S_{gj}}{\sum_g \sum_j S_{gj}} + \mu \frac{\sum_i V_{gi}}{\sum_g \sum_i V_{gi}} \right)$$

where  $R$  is a uniform random variate from 0 to 1 (the same for each genotype),  $L_{\max}$  is the maximum density of establishment (2000 seedlings/100 m<sup>2</sup> by default), and  $\kappa$ ,  $\lambda$ , and  $\mu$  are the proportions of local seed, background seed, and vegetative propagules in the established cohort. These proportions sum to one.

The random variate accounts for absolute differences in propagule input, variation in interspecific competition, and differences in habitat suitability.

### *Assumptions and Limitations*

1. *Seeds are always available for establishment.* This assumption is contingent upon the value of  $\lambda$ , the proportion of establishment that derives from the background seed cloud. In cases where  $\lambda = 0$ , if there is no local seed input, there is no seedling establishment. However, for  $\lambda > 0$ , or when there is at least some local seed input, establishment varies randomly with respect to the number of local seeds that arrive at the site. In reality, there is probably a relationship between establishment and propagule input. However, poplar is a highly fecund and vagile species, and it is a prominent feature of the modeled landscape. Therefore, we expect factors other than propagule input to be drivers of establishment. This is supported by our data from establishment plots, in which there was no consistent relationship between number of seedlings establishing and seed input (Figure 2.19).
2. *Conventional plantation trees behave like wild trees.* This simplification is dictated by the computational difficulties of simulating an added level of genotypes. It is unclear if altered competitiveness of conventional plantation trees would have a significant impact on gene flow from sympatric transgenic plantations. This would depend on a number of interacting factors, including flowering phenology of plantations relative to wild trees, area and location of plantation and wild stands, and availability and location of establishment sites. Incorporation of additional genotypes would be a logical extension to this model in future studies.

## **Competition and Mortality**

### *Primary Data*

Data on density-dependent mortality came primarily from the same experimental plots that provided data on establishment. We followed the fate of seedlings over two to three year periods in 29 plots at two sites (DiFazio *et al.* 1999).

In addition, we drew upon data from a total of 18 field studies in which growth and survival data were gathered for transgenic trees and controls. These trials were intended

to assess the effects of traits such as resistance to the herbicides glyphosate and glufosinate, leaf beetle resistance (*cry3A*), floral sterility, expression of a putative broad-spectrum disease resistance gene (bacterio-opsin), and expression of a variety of selectable and visible marker genes (Meilan et al 2000a; Meilan et al 1999; Meilan et al 2000b; Strauss et al 2001b). These studies provided growth and survival data under selectively neutral conditions (with respect to the transgenic trait), and, where appropriate, under a selective regime that should favor the transgenics (*e.g.*, with herbicide spraying for herbicide resistant trees, and with insect attack for insect-resistant trees).

#### *Implementation in the Model*

The relative competitiveness of transgenics controls the rate at which transgenic and nontransgenic trees die during density-dependent mortality (*i.e.*, self-thinning). Competitive effects of transgenes are simulated through effects on size (see basal area calculation), and density-dependent mortality. This is similar to the Lotka-Volterra equation for two-species interactions (*e.g.*, (MacArthur and Levins 1967; Shugart 1998), except the competitive differential of one genotype is the exact opposite of that of the alternate genotype. In addition to having strong direct effects on competition and mortality, the competitiveness parameter indirectly affects seed and pollen production, which depend on basal area.

Mortality of conventional trees is

$$M_0 = N_0 \left( \left( \frac{N_0 + (1 + \alpha)N_1}{N \max_a} \right) - 1 \right)$$

and mortality of transgenics is

$$M_1 = N_1 \left( \left( \frac{N_0(1 - \alpha) + N_1}{N \max_a} \right) - 1 \right)$$

where  $\alpha$  is a competitive differential,  $N_g$  is the number of trees of genotype  $g$  that are present in the cohort, and  $N_{max_a}$  is the carrying capacity of a cell for age  $a$  (previously defined).  $\alpha$  is the relative difference in growth or resource acquisition of transgenic trees relative to average trees (or half the difference between transgenic and conventional trees) (Figure 2.20). This value can be fixed at initiation, and/or altered in response to selective pressures such as insect herbivory (see Insect Resistance section, Chapter 4), or herbicide application (see Herbicide Tolerance section, Chapter 4).

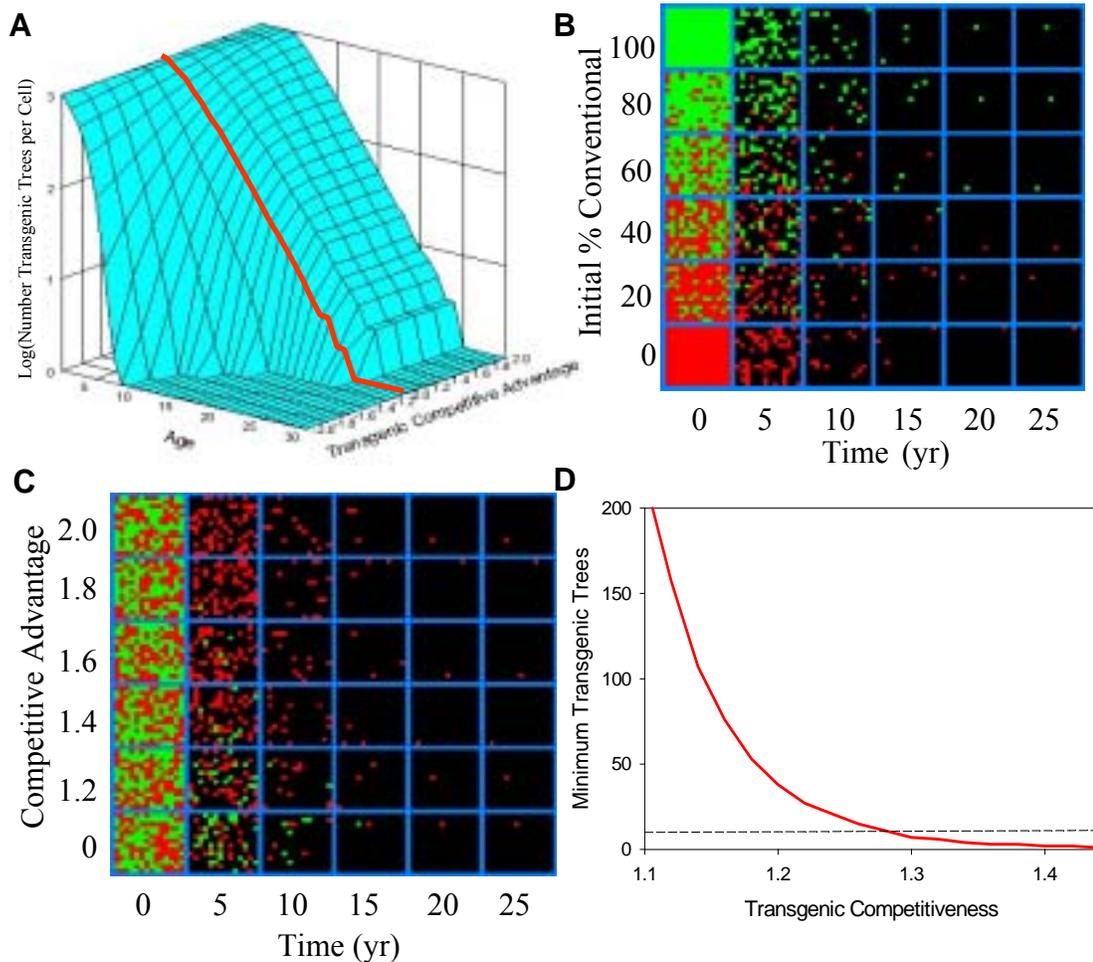
There is no theoretical maximum value for  $\alpha$ , but effective maxima and minima are determined by the rate at which one of the genotypes declines to 0 in the cohort. Density-dependent mortality occurs until a single tree occupies the cell, and that tree persists until eliminated by stochastic disturbance (see disturbance section). If transgenic and conventional trees decline to 0 in the same year, we randomly select a genotype to occupy the cell.

Sex ratio is recalculated each year for each genotype, and equal numbers of male and female trees die initially. When there are 100 or fewer trees, we determine the number of males of each genotype that will die by sampling from a binomial distribution in which each dying tree has a probability of 0.5 of being male. This allows the chance development of skewed sex ratios.

### *Assumptions and Limitations*

1. *Carrying capacity is constant across the landscape and through time.* We do not vary the maximum number of trees that can occupy a site, though the number of trees that actually become established is varied randomly. In fact, factors such as interspecific competition, microclimate, soils, and disturbance will cause variation in the carrying capacity and mortality rates at different sites, and these factors could change through time. However, poplar typically occurs as relatively pure stands in the study area at the scale of a single cell (100 m<sup>2</sup>), and our approach is to average out site-specific variation across the landscape. Also, there is unlikely to be tremendous variation in carrying capacity over the typical duration of our model runs (50 years), unless the hydrologic regime is drastically altered.

2. *Transgene fitness effects are manifested through growth rate, intraspecific competition, and fecundity.* We do not allow for competitive differences at potentially key selective stages such as pollination or establishment. However, fecundity differences can be interpreted as integrating factors such as pollen and



**Figure 2.20** Competition in the STEVE model. **A.** Number of transgenic trees per cell versus age of the cohort and the competition differential. The initial cohort consists of 1000 transgenic and 1000 conventional seedlings, and density-dependent mortality results in a single tree in the cell by age 25. The red line indicates the trajectory under neutral fitness. Trajectory for conventional trees is indicated by trend of opposite sign (e.g., for competition differential 1.1, conventional tree trend is indicated by  $-1.1$  line). **B.** Representation of transgenic (red squares) and conventional (green squares) seedlings in plots through time at different starting densities with neutral fitness. The eventual occupant of the cell is determined by the starting density. **C.** Influence of competitive differential on cell occupancy, with equal starting densities. Transgenics take over the cell more rapidly as competitive differential increases. **D.** Minimum number of

transgenic trees required at establishment for eventual elimination of conventional trees from cohort at different levels of transgenic advantage. Cohort initially contained 2000 total trees at establishment. A single tree remained after 25 years of density-dependent mortality. Broken line represents minimum number of transgenic seedlings present at each establishment site in baseline sensitivity analysis scenarios. Intersection of lines is threshold competitiveness value for maximum transgenic advantage.

ovule production, pollen viability, pollen tube growth rates, and embryo development. Also, density-dependent mortality following germination is quite intense in the early years of the cohort, and competitive effects can be almost immediately apparent (Figure 2.20). Therefore, differences in establishment ability can be incorporated into the density-dependent mortality competition coefficient.

## **Plantation Management**

### *Source Data*

Plantation management occurs following the general practices of forest industries that grow hybrid poplar in the study area. Plantations are managed in large (mean = 16 ha) single-clone blocks in an even-aged fashion, so that approximately the same area is harvested each year.

### *Implementation in the Model*

Trees are harvested upon reaching rotation age, which is set at model initiation. New plantations are then established, with genotype and sex being determined with probabilities set at model initiation.

### *Assumptions and Limitations*

1. *Plantation management does not change for the duration of model runs.* Total plantation area, average area of transgenics, rotation length, and sex ratio are predetermined at initiation. In reality, plantation management can vary markedly over fairly short periods in response to market forces or changes in management personnel or philosophy. In fact, during the course of this project, standard industrial rotations in the region changed from 8 years to 12 years, and density declined from 2400 trees/ha to 1200 trees/ha. For simplicity, we simulate different management regimes in separate model runs. This approach allows for a clearer assessment of the impacts of management practices on potential gene flow.

## Sensitivity Analysis

One of the key features of the model is it allows us to perform sensitivity analyses, which consist of ‘virtual experiments’ in which we vary individual parameters, holding all else constant at reasonable values, and assess changes in model outcomes (Haefner 1996). Sensitivity analyses require a very large number of model runs, and therefore substantial computing power. To speed processing and allow more model runs, we devised a test landscape which contained many of the features of the real landscape, but on a much smaller scale (full landscape = 46,000 ha, test landscape = 2,500 ha). Important characteristics such as habitat types and poplar edge:interior ratios were similar between the test landscape and the full landscape (Figures 2.21A and 2.21B). For the bulk of the sensitivity analyses, we explored a scenario with a very high component of poplar plantations on the landscape, 50% of which were transgenic (Figure 2.22A). In addition, we assessed transgene flow on a landscape containing a single small plantation (19 ha), which simulates a large, isolated field trial (Figure 2.22B).

Using the test landscapes, we analyzed the effects of 30 variables and a variety of interactions, analyses that required more than 8000 model runs of 50 years each. For the sensitivity analyses we set all parameter values at a default baseline condition (Table 2.8). These conditions are hypotheses, and the sensitivity analyses depict the consequences of deviating from this condition. We varied selected parameters individually and assessed changes in model outcomes for 10 repetitions of each scenario<sup>5</sup>. Each repetition was initialized with a different random number, so stochastic processes such as establishment and mortality varied in space and time for each repetition.

The response for the sensitivity analyses was the ‘mean area of mature transgenics’, which is the percentage of area of mature poplar occupied by transgenic trees outside of plantations (*i.e.*, wild poplar populations). This response was averaged over the final 25 years of the run to dampen interannual variability and simplify presentation of results. Time trends for individual scenarios were generally concordant with trends of the 25 year means (*e.g.*, Figures 2.23A and 2.23B). We chose this response value on the assumption that the area of mature transgenics best represents ecologically significant gene flow.

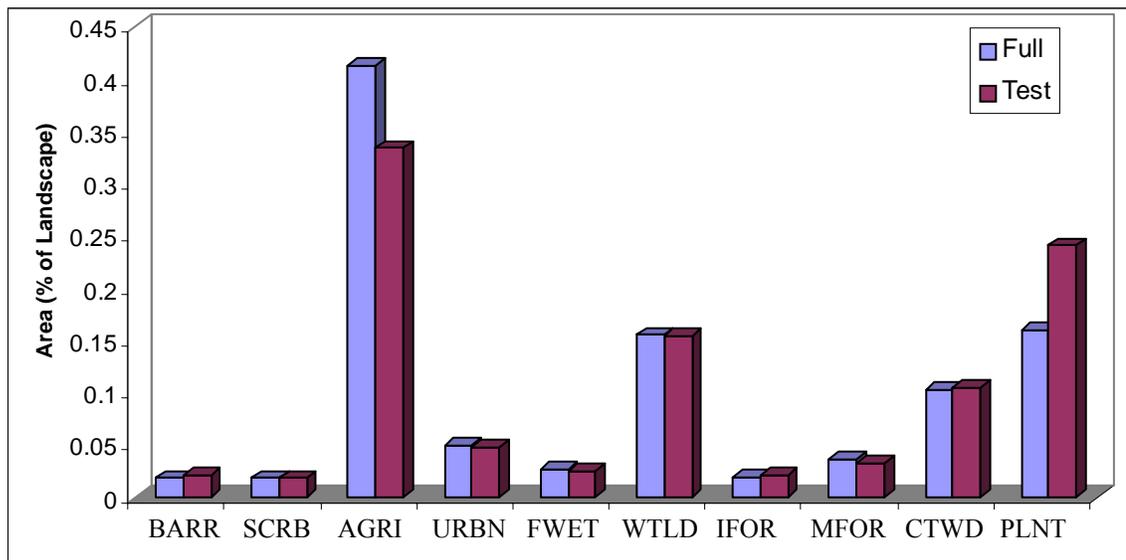
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<sup>5</sup> For the purpose of this thesis, a scenario is defined as a unique combination of parameter values

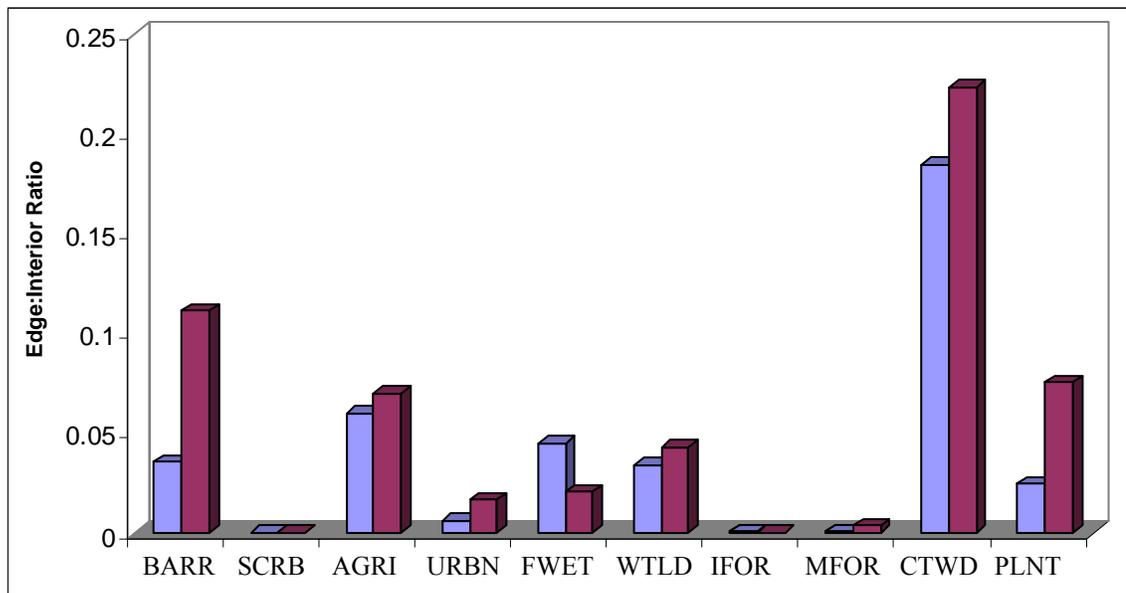
**Table 2.8** Baseline conditions for sensitivity analyses.

<b>Parameter</b>	<b>Value</b>
Landscape Area	2500 ha (500 x 500 pixels)
Plantation Area	485 ha (19.4% of landscape)
Transgenic Plantations	240 ha (9.6% of landscape)
Plantation Rotation	12 years, even aged
Plantation Density	1500 trees/ha
Plantion Sex Ratio	50% female, by area
Transgenic Fertility	1 (fully fertile)
Transgenic Competitive Advantage	0
Initiation of flowering, plantations	5 years
Initiation of flowering, wild	10 years
Pollen Dispersal	Neighborhood, 440m,50%; slope, -0.007; intercept, 0.67;
Seed Dispersal	Neighborhood, 220m,90%; slope, -0.05; intercept, 0.9;
Vegetative Dispersal	Neighborhood, 220m,100%; slope, -0.1; intercept, 0.6;
Phenology Classes	1 (all trees fully compatible)
Wind	No influence of wind on dispersal
Maximum Establishment Density	20 seedlings/m <sup>2</sup>
Density-Dependent Mortality	Slope, -0.33
Basal Area Increase	Slope, 0.04
Establishment Rates	Empirical (Figure 2.7)
Maximum Establishment, Agricultural Edges	0.05 ha

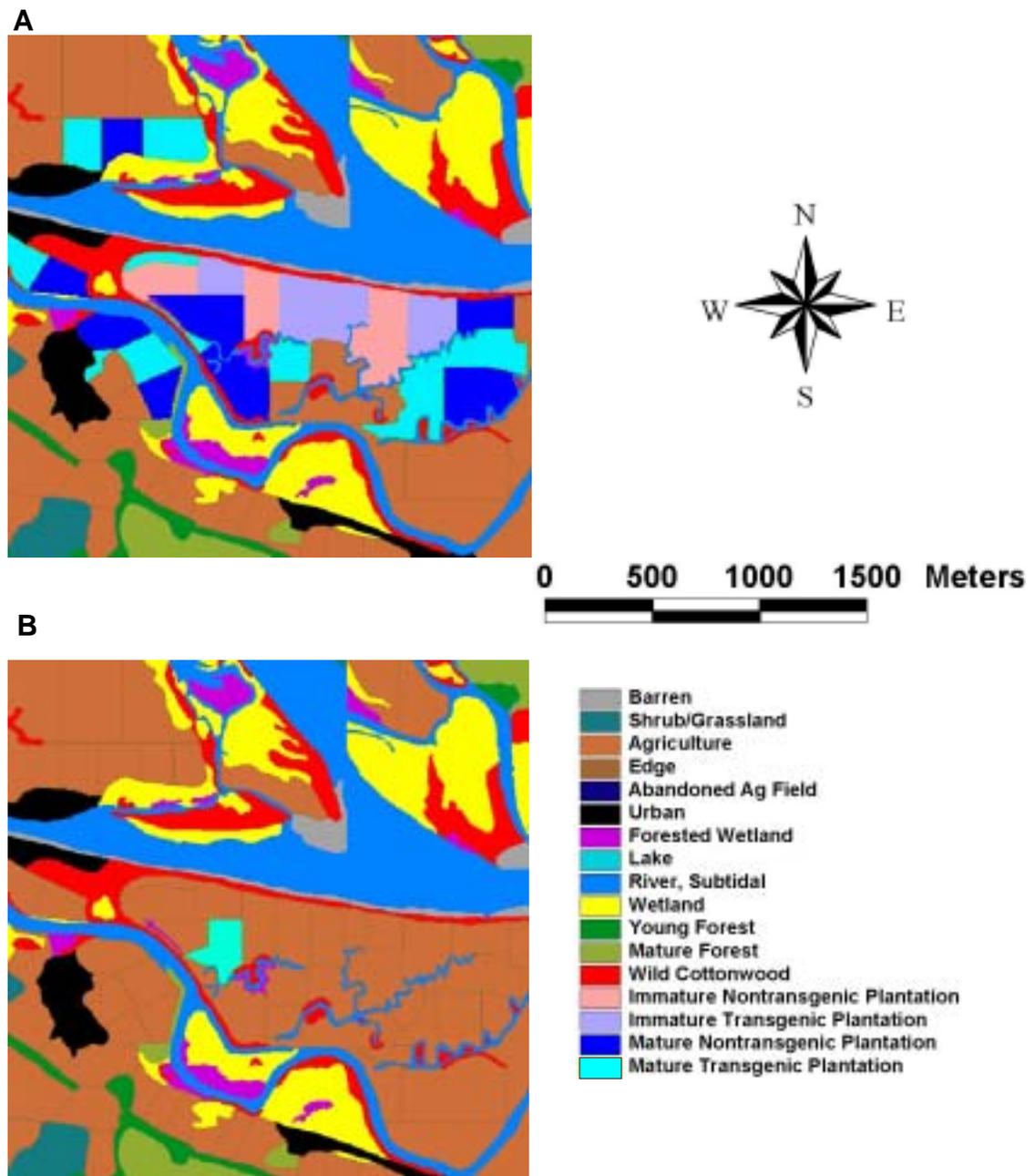
A



B



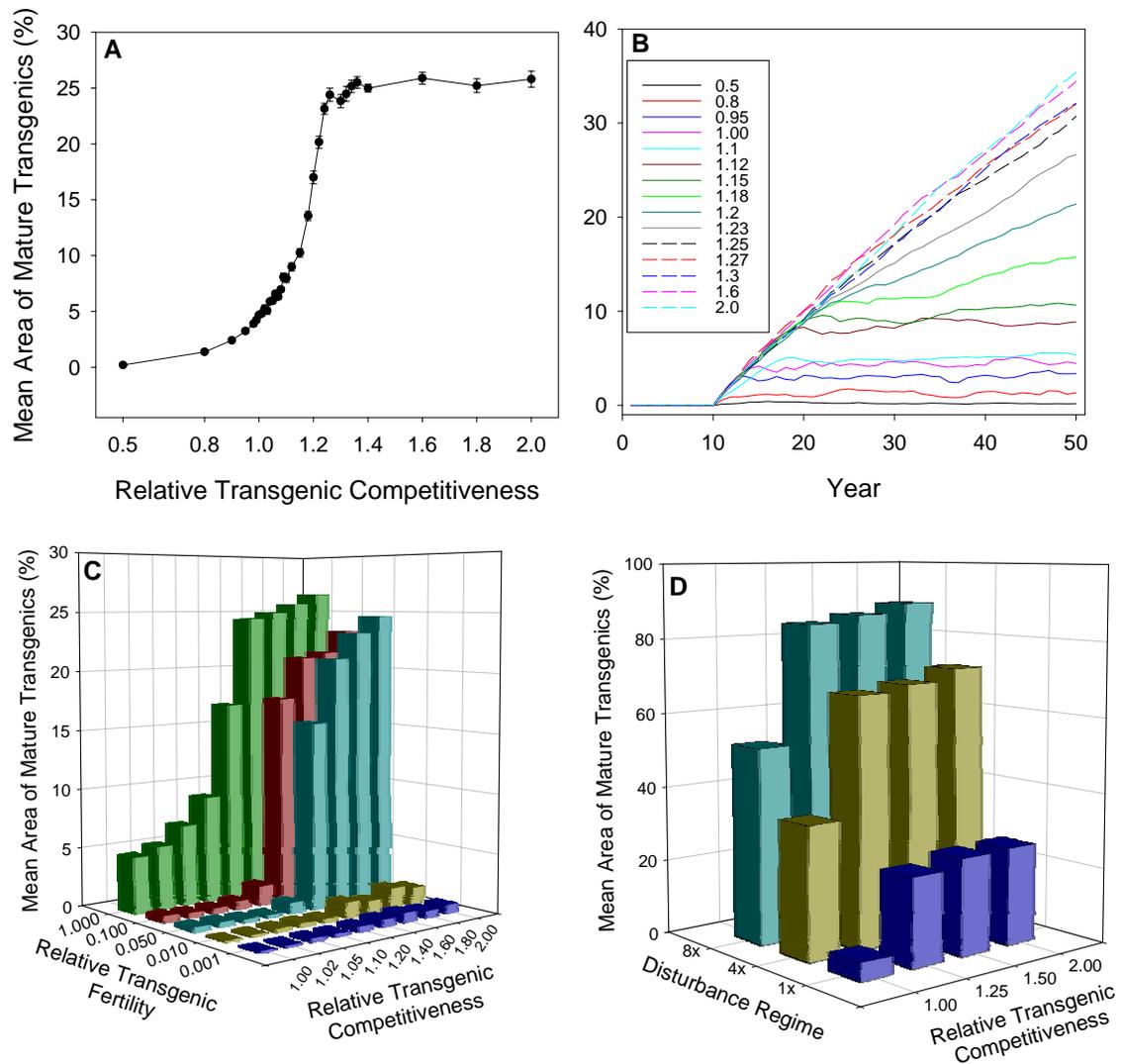
**Figure 2.21** Comparison of characteristics of test landscape with those of full landscape. **A.** Proportion of landscape occupied by each cover type, relative to total area available for poplar establishment (*i.e.*, excluding river and high elevations). Test landscape has a slightly higher proportion of agricultural land in poplar cultivation than the full landscape. **B.** Poplar edge:interior ratio. Poplar edges are those cells that are at the edge of a poplar polygon. The test landscape has a higher proportion of cells at poplar edges, partly because of the preponderance of linear poplar polygons along the river (Figure 2.22). Habitat types are defined in table 2.1.



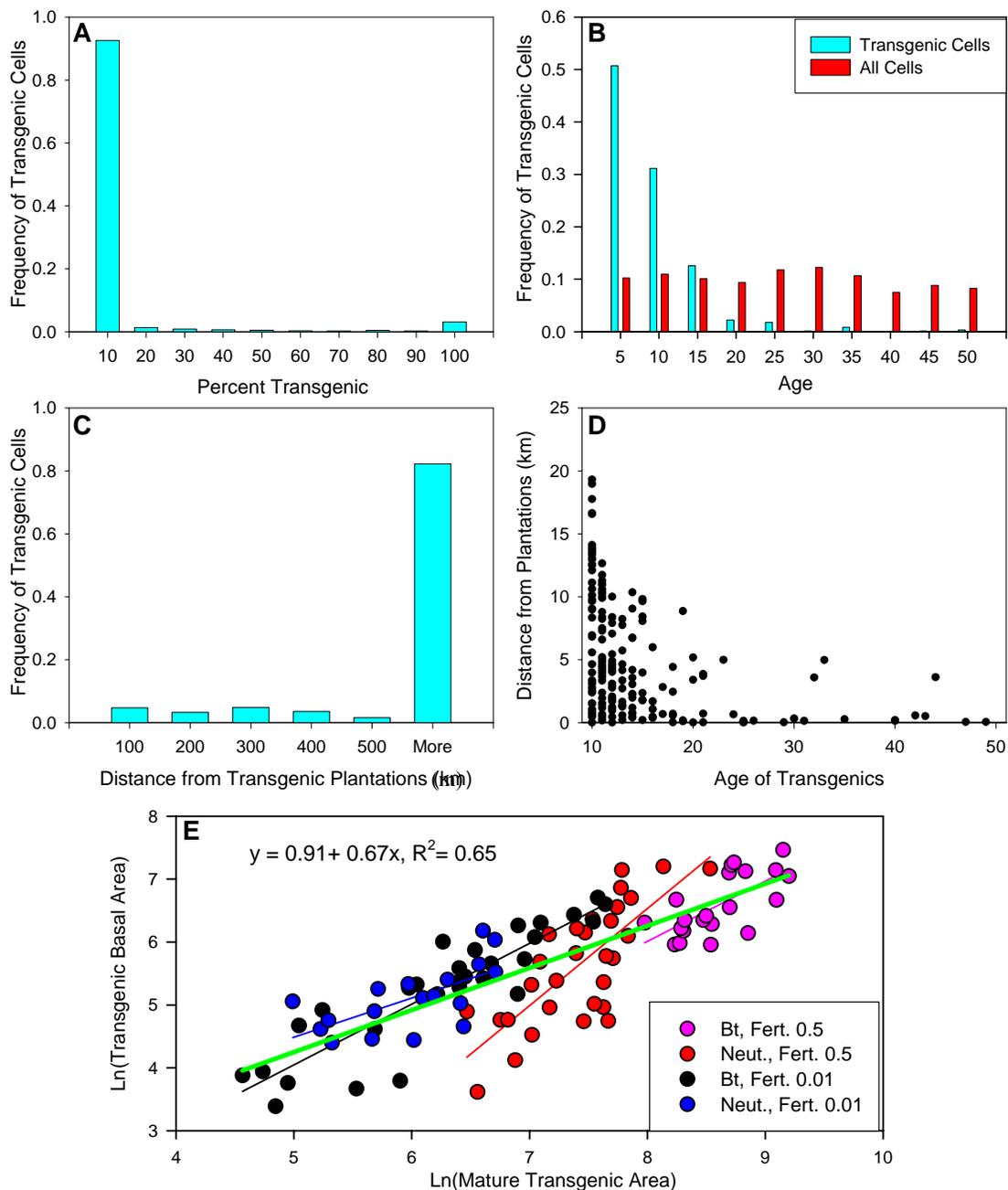
**Figure 2.22** GIS layers depicting cover types for two synthetic landscapes used in the sensitivity analyses. Original layers were created by digitizing air photos of the lower Columbia River. The test landscapes were derived by combining elements of the larger landscapes so that essential features of the large landscape were retained in the smaller area, thus allowing a vast increase in the number of model runs. **A.** Commercial cultivation scenario. **B.** Field trial scenario

Young transgenic trees may be destined to be eliminated by competition before they attain a stature that would allow significant ecosystem effects. For most model runs, this response reached an apparently stable equilibrium within the 50 year runs, indicating that it is a good indicator of long-term levels of transgene flow under modeled conditions (Figure 2.23B). It is also important to note that the grain of the STEVE model, 100 m<sup>2</sup> cells, is rather coarse in that multiple trees may occupy a single cell until the age of 25 (Figure 2.4). Therefore, the area of mature transgenics can be misleading because a cell is considered ‘occupied’ even if transgenics represent a minority of the trees present in the cell. In fact, in a typical scenario with neutral transgenes, transgenic trees constituted a minority of trees in most cells (Figure 2.24A), and representation declined with age (Figure 2.24B). Most of the established transgenics derived from background seed flow (91%), as demonstrated by the long distances between transgenic cohorts and plantations (Figure 2.24C). These background transgenics largely disappeared from the landscape as they were eliminated by density-dependent mortality, and older transgenics tended to be closer to plantations (Figure 2.24D). Therefore, the influence of transgenics would likely be minor for much of the area we are considering to be ‘occupied’ by transgenics with our transgene flow estimate. Basal area is probably a more accurate measure of the potential influence of transgenics in the wild, because this integrates age (size) and density. However, basal area was linearly related to mean area of mature transgenics across a broad range of values (Figure 2.24E). Therefore, our measure of transgene flow reveals the same general trends as basal area.

Variation of individual parameters and selected combinations provides a great deal of information about the factors controlling transgene flow in our model. However, it is likely that many of these parameters interact, such that changing the value of one parameter alters the effects of other parameters. We therefore sought to explore the effects of the most important parameters in a fractional factorial experiment. Fractional



**Figure 2.23** Effects of relative transgenic competitiveness on transgene flow. **A.** Effect of competitiveness on transgenic gene flow, as measured by the percentage of area of mature wild cottonwood that contained transgenic trees, averaged over years 25-50 of the simulation. Relative competitiveness can be interpreted as the number of normal tree equivalents represented by one transgenic tree. Bars represent standard errors of 10 repetitions (each with a different initializing random number). Relative competitiveness has a strong effect on transgene flow in the range of 1 to 1.25, after which the effect reaches an asymptote. **B.** Time course of selected individual scenarios plotted in A. Above a competitiveness value of 1.15, transgene flow continues increasing up to year 50 and beyond, whereas transgene flow reaches a steady state by year 25 for lower competitiveness values (as is the case for most scenarios). **C.** Interaction between transgenic competitiveness and transgenic fertility. Transgenics with reduced fertility had greatly reduced gene flow, even when competitiveness is implausibly high. Vegetative propagules largely account for the low levels of gene flow observed for highly sterile trees. **D.** Interaction between competitiveness and disturbance regime. The asymptote in B is not observed under greatly enhanced disturbance.



**Figure 2.24** Characteristics of cells containing transgenic trees. Data for A,B,D, and E for 5 repetitions of 50 years for full landscape with neutral transgenic fitness and transgenic fertility of 0.5 (baseline for risk assessment simulations, Chapter 4). **A.** Percentage of transgenic trees in wild poplar cohorts. **B.** Age distribution of cohorts containing transgenics compared to overall age distribution for established poplar. **C.** Distance of transgenic cohorts from mature transgenic plantations **D.** Distance from mature transgenic plantations (at time of establishment) and age of transgenic cohorts. **E.** Relationship between area of mature transgenics and transgenic basal area for a trait with neutral fitness (Neut.), and insect resistance (Bt) and with fertility of 0.5 and 0.1. Lines are linear regressions for each simulation; green line and equation for all data combined.

factorial experiments allow exploration of main effects and selected levels of interactions by using higher level interactions as aliases for the level of interest, indicated by the resolution of the design. This allows exploration of interactions of a large number of factors with a modest number of scenarios. We performed a resolution V fractional factorial with 11 factors at two levels per factor. A resolution V design allows discrimination of all main effects and two-factor interactions. This required 128 scenarios, with 5 repetitions per scenario (Box *et al.* 1978). We performed this analysis for a landscape with commercial-scale transgenic plantations (Figure 2.22A) and a transgenic field trial (Figure 2.22B). We chose factors that had a substantial effect on gene flow in single-factor analyses (competitiveness, disturbance, fertility, phenology, plantation reproductive maturity, and distant seed establishment), or for which interactions with important parameters were deemed likely (plantation sex, vegetative establishment, vegetative dispersal). We chose two levels for each parameter, representing extremes of a reasonable expected distribution of values (Table 2.9). Where possible, we chose the lowest value of a parameter that caused a substantial response in single-factor analyses (*i.e.*, where the response began to reach an asymptote). Significance was assessed based on standard F-statistics ( $P < 0.05$  of observing a larger F-value).

The fractional factorial analysis was potentially susceptible to experimenter bias because only two levels were examined for each factor, and the levels were chosen somewhat subjectively to reflect reasonable values that could have a strong influence on gene flow. We explored the robustness of the results by repeating the experiment with factors varied +/- 20% from our best estimates of parameter values (Table 2.9).

## **Results**

### **Fertility**

As expected, fertility of transgenic trees had a strong effect on minimizing gene flow from transgenic plantations. With highly reduced fertility, gene flow was among the lowest levels observed for all scenarios: between 0.1 and 0.2%, compared to approximately 5% gene flow for fully fertile transgenics. In addition, transgene flow

**Table 2.9** Parameter values used in fractional factorial analyses. ‘Biological Range’ was selected based on biologically reasonable upper and lower estimates for parameters. Factors were varied +/- 20% from initial parameter estimates for ‘Objective Range’.

Parameter	Abbrev.	Biological Range		Objective Range	
		Lower	Upper	Lower	Upper
Fertility	FER	0.01	1	0.4	0.6
Transgenic Competitiveness	COMP	0.99	1.2	0.8	1.2
Plantation Maturity	PLANT	5	8	4	6
Phenology	PHEN	25% compat. <sup>a</sup>	65% compat.	52% compat.	82% compat.
Disturbance	DIST	1x(-15%) <sup>b</sup>	3x(+15%)	1x(-15%)	3x(+15%)
Distant Pollination <sup>c</sup>	POLCLD	10%	50%	40%	60%
Distant Seed Establishment <sup>d</sup>	SDCLD	1%	10%	8%	12%
Vegetative Establishment	VEG	1%	40%	8%	12%
Vegetative Dispersal Slope	VGD	-0.05	-0.1	-0.08	-0.12
Plantation Sex <sup>e</sup>	SEX	0	0.5	0.4	0.6
Rotation	ROT	8	12	8	12

<sup>a</sup> Phenology is expressed as compatibility with wild trees relative to a case with one phenology class (complete overlap in flowering among all trees)

<sup>b</sup> Disturbance rates were selected based on changes in wild poplar populations. Empirical disturbance rates resulted in a 15% reduction in poplar populations over a 50 year simulation. Enhancing establishment 3-fold resulted in a 15% increase in wild poplar over 50 years.

<sup>c</sup> Distant pollination is proportion of seeds that are fathered by nonlocal males (determined by total proportion of pollen produced on landscape)

<sup>d</sup> Distant seed establishment is proportion of seedlings derived from nonlocal seeds

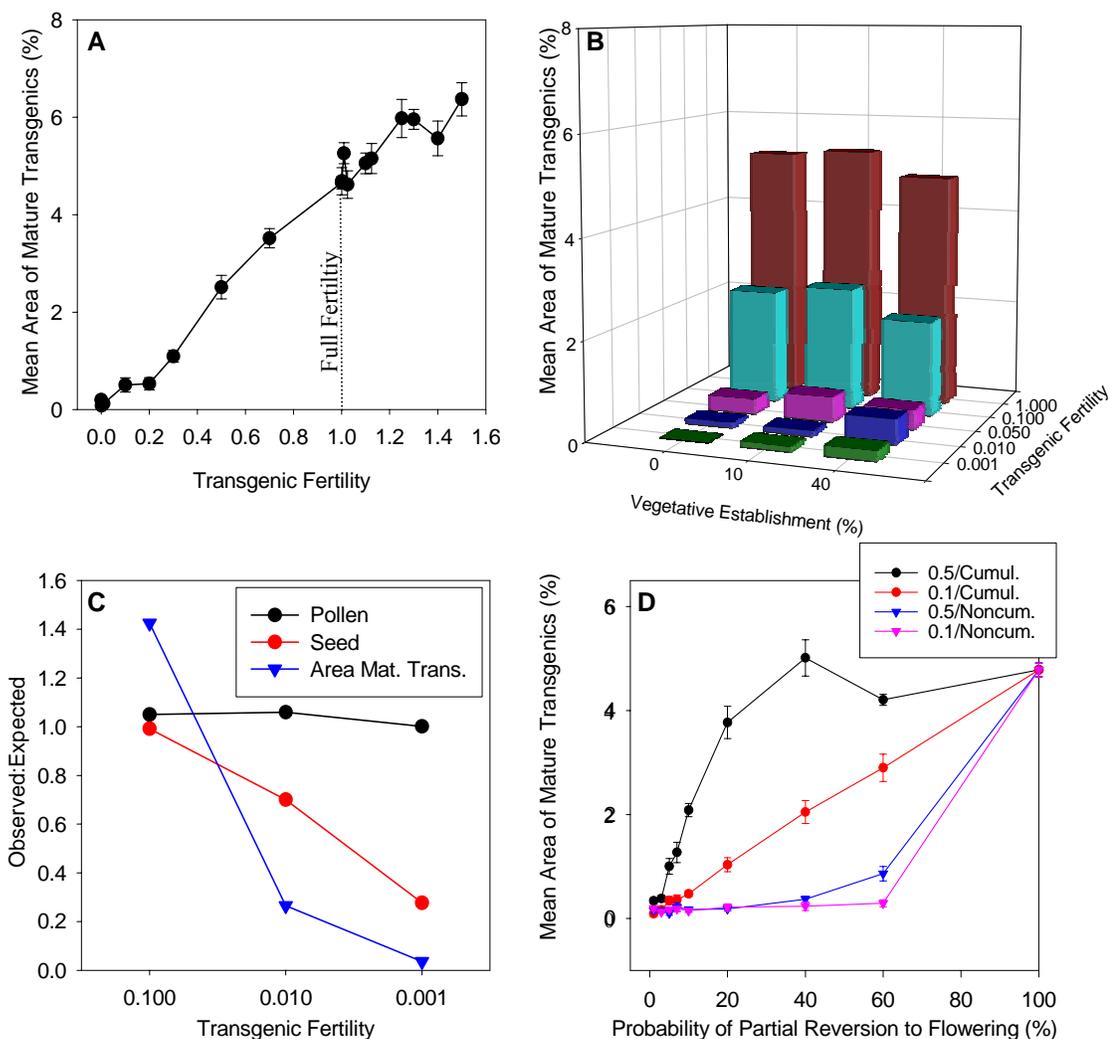
<sup>e</sup> Ratio of male to female plantation blocks

rates were not distinguishable for fertility ranging from 0 to 1% of wild fertility, indicating that complete sterility was not required to attain maximum gene containment (Figure 2.25A).

The low level of gene flow for fully sterile plantations was due to movement of vegetative propagules in the vicinity of plantations. Little is known about vegetative dispersal and establishment in wild poplars. However, transgenic gene flow remained very low under a wide range of rates of vegetative establishment (Figure 2.25B), and gene flow rates were relatively insensitive to changes in rates of vegetative establishment and shapes of vegetative dispersal curves (Figures 2.25B, 2.26A and 2.26D). Sexual fertility therefore was much more important in controlling gene flow.

Nearly 50% of the gene flow with low fertility transgenics (Fertility < 0.1) was due to sexual reproduction, as demonstrated by simulations with vegetative establishment eliminated (Figure 2.25B). Pollen production decreased proportionally with fertility, but seed production and gene flow were relatively insensitive to reduction in fertility below 0.1 (Figure 2.25C). Transgenic seed production shifted to conventional plantation trees as fertility was reduced, until 96% of transgenic seeds were produced by conventional plantation trees when transgenic fertility was 0.0001 (Table 2.10).

In some scenarios, individual trees were allowed to revert to partial fertility with a range of probabilities. We made a liberal assumption that fertility would be restored to 50% of wild fertility for each reversion event (sampled from a normal distribution), and reversion accumulated over multiple years (*i.e.*, the effect was permanent). A 20% probability of reversion was required for gene flow levels to approach those of fully fertile trees. With a restoration level of 10%, gene flow was considerably less than full fertility, even with reversion rates as high as 60%. Gene flow with reversion rates up to 3% was indistinguishable from that of trees with stable sterility. If reversion was not cumulative (*i.e.*, fertility reset to 0.01 each year for each tree), gene flow was still greatly reduced compared to wild trees, and was marginally greater than for trees with stable sterility. These results were manifested across a broad range of probabilities of reversion (Figure 2.25C).



**Figure 2.25** Effects of transgenic fertility on transgene flow. **A.** Effects of fertility of transgenic trees relative to nontransgenics. **B.** Interaction between vegetative establishment and fertility. Vegetative establishment is the proportion of established individuals in a new cohort that are derived from vegetative propagules. Variation in vegetative establishment had little overall effect on transgene flow, although a minor effect is apparent at low levels of fertility. **C.** Effect of reduced fertility on pollen production, seed production, and transgene flow (Area Mat. Trans.) relative to fully fertile transgenics. Relative effect calculated as ratio of change in response to change in fertility level. Values of one indicate a directly proportional response. **D.** Effects of unstable sterility on transgene flow. Probability of sterility breakdown is the probability of a reversion to flowering. If the probability is exceeded, fertility is restored with a mean of 0.1 or 0.5, sampled from a normal distribution with a standard deviation of 0.05 or 0.25 respectively. Reversion was cumulative (Cumul.) for each tree through time, or fertility was reset to the base value annually (Noncum.). Low values of instability had little effect on gene flow, and a reversion rate of about 20% would be required for gene flow levels to approach those of fully fertile transgenics.

**Table 2.10** Proportion of transgenic seeds produced by plantation transgenics, plantation conventional trees, and wild trees under a range of fertility values. Transgenic seed production was measured in the first year of simulations on the test landscape with 50% of plantations conventional (default condition), means of 10 repetitions.

<b>Fertility</b>	<b>Transgenic Plantation (%)</b>	<b>Conventional Plantation (%)</b>	<b>Wild (%)</b>
0.0001	1.3	96.4	2.2
0.001	9.0	75.7	15.3
0.01	21.6	41.5	36.9
0.1	25.5	31.8	42.7
0.5	26.6	30.4	43
1	27.3	30.3	42.4

## Competitiveness

As expected, relative competitiveness had substantial effects on gene flow. On the one hand, transgenics with competitiveness values<sup>6</sup> of 0.5 (a severe growth disadvantage in the wild), had gene flow rates comparable to those of partially sterile trees (0.1%). On the other hand, enhanced competitiveness greatly accelerated gene flow (Figure 2.23A), and competitiveness values above 1.18 resulted in a continual increase in transgenic trees with no sign of abatement (Figure 2.23B), even after 100 years of simulation (not shown). The effects of enhanced competitiveness reached a saturation point at about 1.25 (25% growth advantage) (Figure 2.23A). The magnitude of this plateau was largely determined by the disturbance regime: effects of a competitive advantage were greatly enhanced under high disturbance (Figure 2.23C).

When we varied transgenic fertility and competitiveness simultaneously, the effects of superior competitiveness were largely obviated for transgenics with reduced fertility.

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<sup>6</sup> We modeled relative competitiveness as differences in growth rate, controlled by a single parameter. Therefore, we express relative competitiveness in terms of equivalents of nontransgenic trees. For example, transgenics with a 20% growth advantage relative to wild trees would have a competitiveness value of 1.2.

This was true even for very high transgenic competitiveness values, and relatively modest fertility reduction (*e.g.*, 1% fertility, Figure 2.23D).

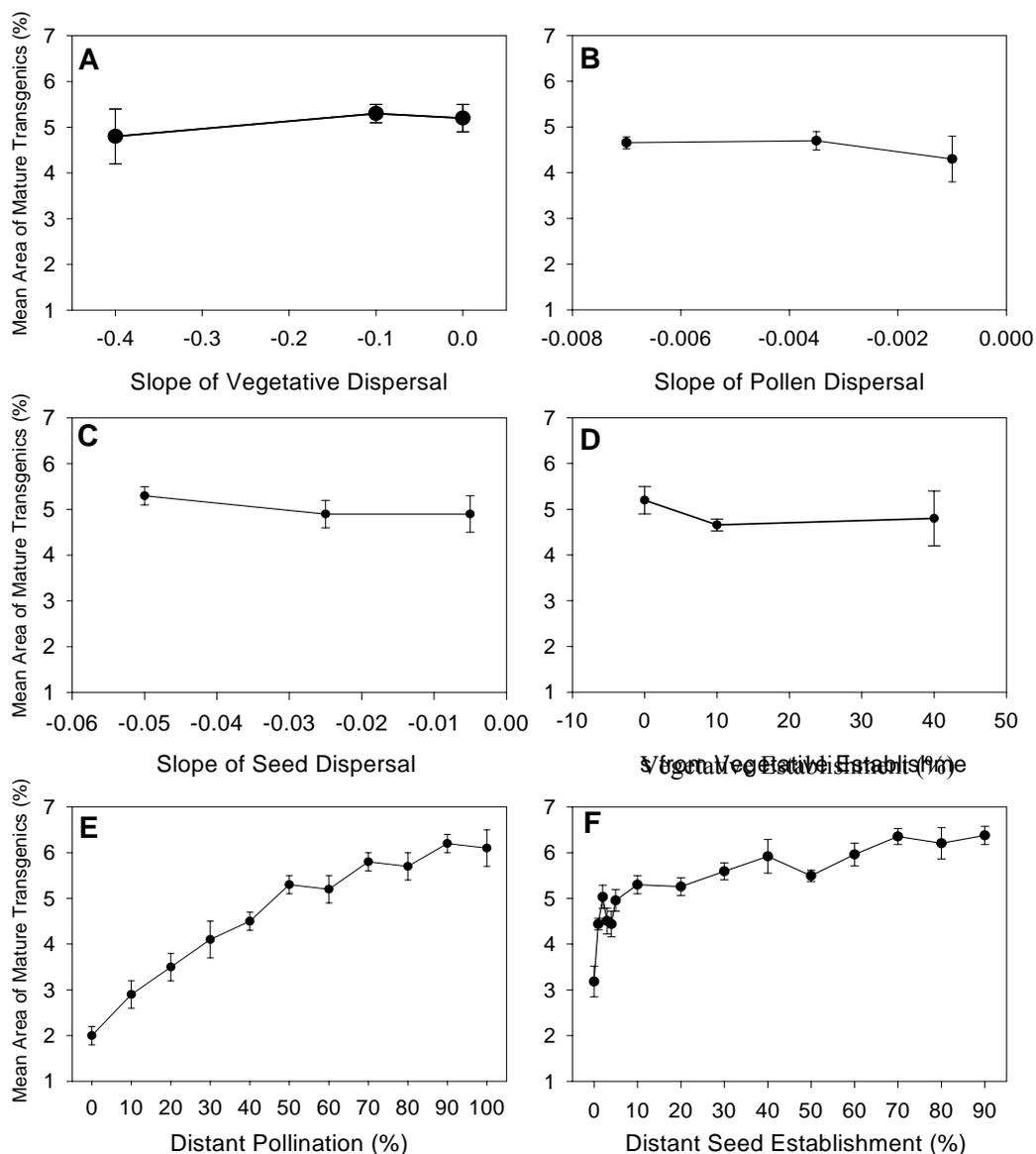
## **Dispersal and Establishment**

Transgene flow was relatively insensitive to variation in the shapes of local distributions of pollen, seed and vegetative propagules (Figures 2.26A, 2.26B, and 2.26C).

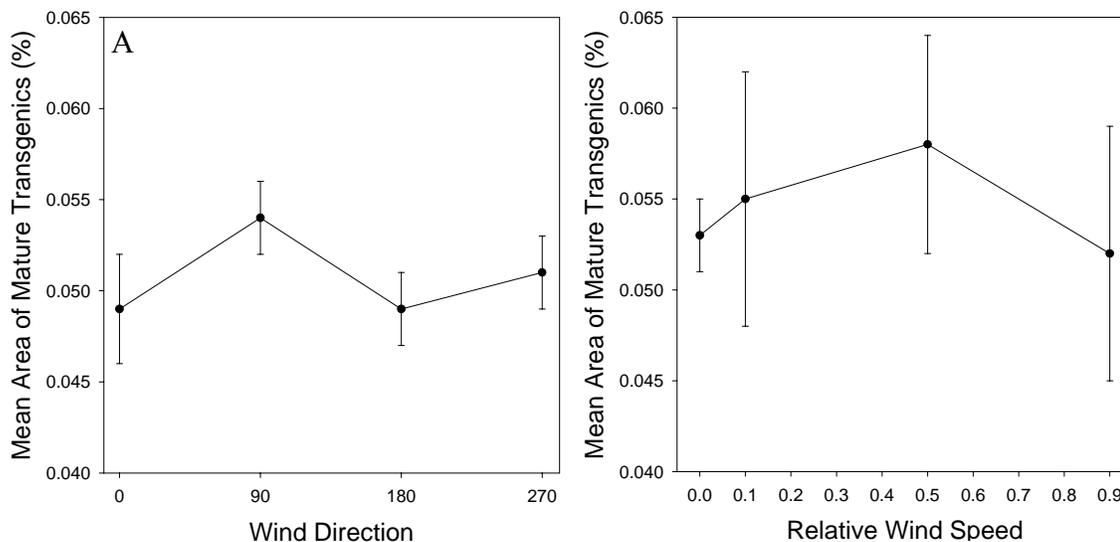
We also examined the effects of varying the amount of pollination from distant sources. This had a strong effect on gene escape in the range of 0 to 50% distant pollination, in which transgene flow more than doubled (Figure 2.26E). Similarly, increasing the amount of establishment due to distant seeds enhanced transgene flow at low levels of distant establishment, though the effect was not as strong as that of distant pollination (Figure 2.26F). In contrast, vegetative dispersal and establishment had little effect on transgene escape (Figure 2.26A,D).

## **Wind**

Neither wind direction (Figure 2.27A) nor relative wind speed (Figure 2.27B) had substantial effects on transgene flow, though there was a marginal increase when wind direction was set at 90°, which is the presumed prevailing wind direction on the lower Columbia River (*i.e.*, from the west).



**Figure 2.26** Effects of dispersal and establishment on gene flow. **A-C.** Effects of varying the slope of the negative exponential distributions depicting local vegetative, pollen, and seed dispersal, respectively. Varying this slope had little effect on gene flow. **D.** Effects of vegetative establishment on transgene flow. Vegetative establishment is the proportion of established individuals in a new cohort that are derived from vegetative propagules. **E.** Effects of distant pollination on transgene flow. Distant pollination is the proportion of seeds that are fathered by trees that do not occur in the local population. This parameter has a strong effect on transgene flow, reflecting the importance of long distance pollen dispersal. **F.** Effects of distant seed establishment on transgene flow. Distant seed establishment had relatively minor effects except at very low levels. Error bars are 1 standard error from 10 repetitions with each set of parameter values.



**Figure 2.27** Effects of wind direction and wind magnitude on transgene flow. **A.** Effect of prevailing wind direction (in degrees), with relative wind speed set at 0.5. **B.** Effect of relative wind speed, with wind direction set at 90 degrees.

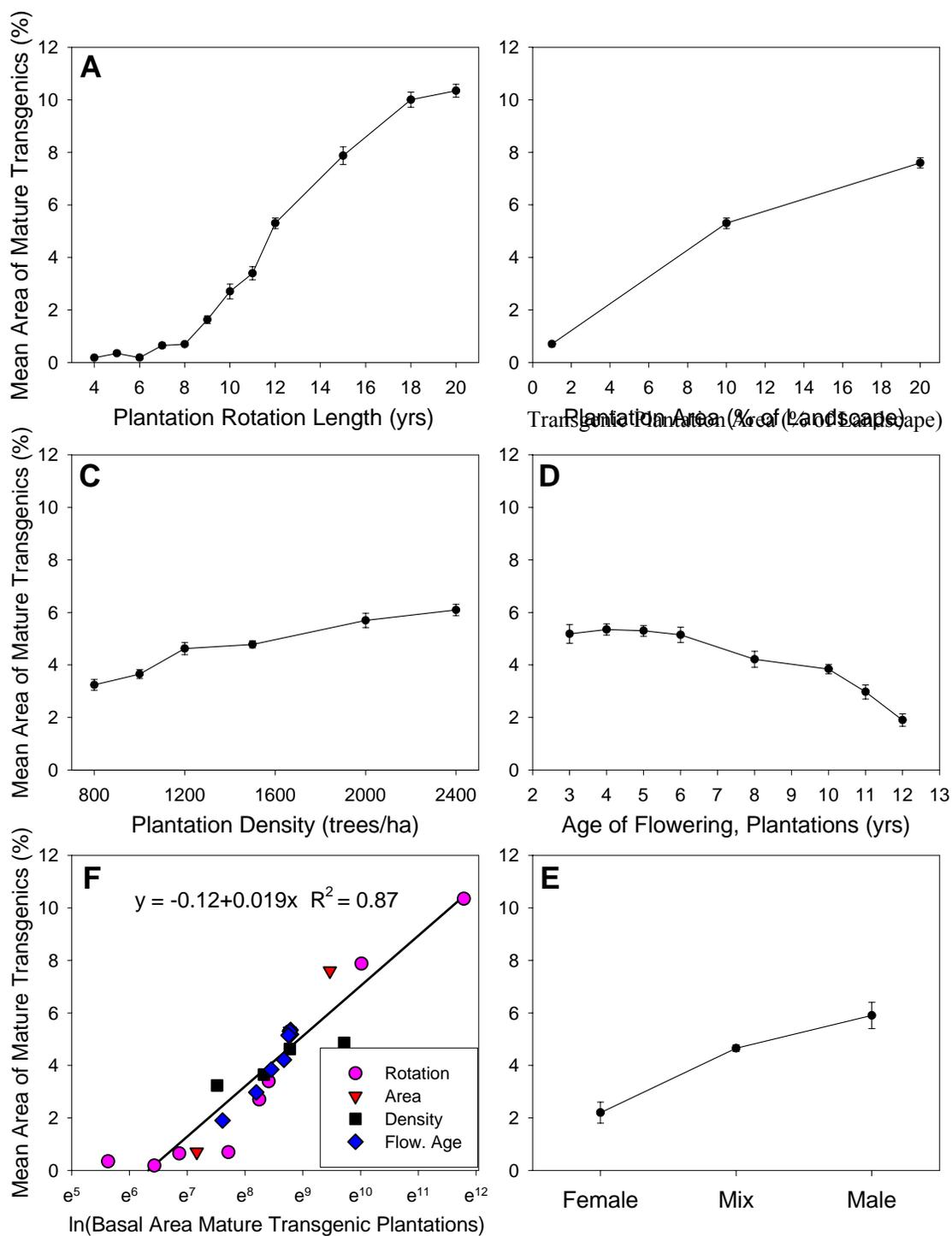
## Plantation Silviculture

Increased rotation length (Figure 2.28A), transgenic plantation area (Figure 2.28B), and plantation density (Figure 2.28C) each caused increased transgene flow. In contrast, increasing the age of plantation flowering led to reduced transgene flow (Figure 2.28D). These effects were closely tied to changes in total basal area of mature transgenic plantation trees, which was strongly correlated with gene flow in these scenarios (Figure 2.28F). Also, gender of clones in plantations had a moderate influence on transgene flow, with female plantations yielding less gene flow than mixed or male plantations (Figure 2.28F).

## Poplar Characteristics

### *Flowering Phenology*

Analyses of the influence of flowering phenology are complicated because effects depend on both the distribution of flowering times for transgenic and nontransgenic trees,



**Figure 2.28** Effects of plantation characteristics on transgene flow. **A.** Plantation rotation. **B.** Area of landscape occupied by transgenic plantations. **C.** Plantation density. **D.** Age of flowering of plantations. **E.** Transgene flow versus basal area of mature transgenic plantations in year 25, with all data combined from scenarios in graphs A-D. Line is least square regression, with a natural log transformation of basal area to correct for heteroscedasticity. **F.** Plantation Gender: all female, 50:50, and all male.

as well as the influence of flowering time on sexual compatibility. If plantation trees were completely phenologically incompatible with wild trees, gene flow was attenuated. However, partial flowering overlap largely overcame this effect, and there was even a marginal enhancement of transgene flow with moderate phenological incompatibility (Figure 2.29A).

### *Carrying Capacity*

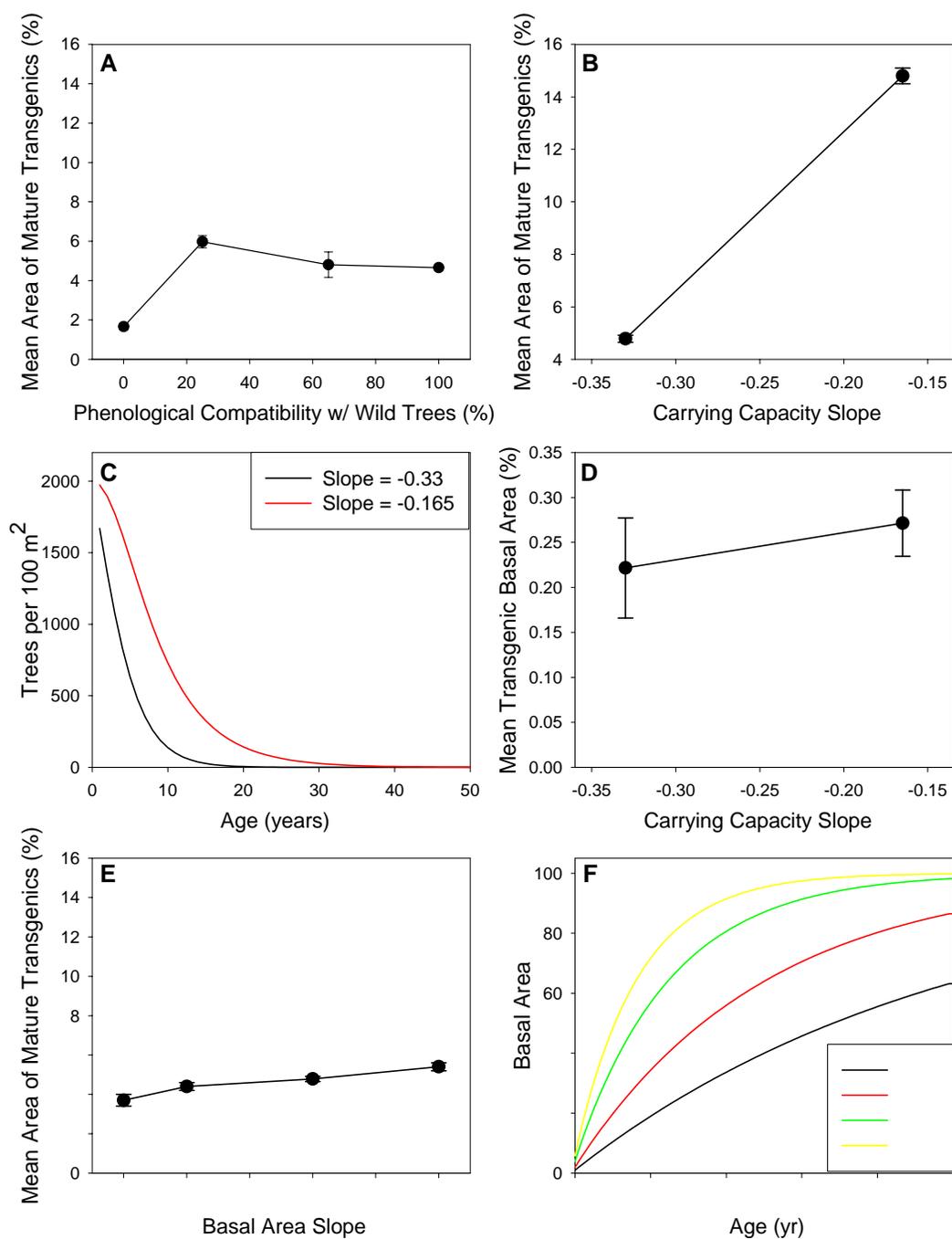
Decreasing the slope of the equation for carrying capacity of establishment sites resulted in an apparent increase in transgene flow (Figure 2.29B). However, this increase was largely transient, as the effect of increased carrying capacity is primarily an extension of the competitive exclusion phase (*i.e.*, the period required for population density to reach 1 tree/m<sup>2</sup>) (Figure 2.29C). If examined in terms of relative basal area, there was no significant increase in transgene flow as a result of increased carrying capacity (Figure 2.29D).

### *Growth Rate*

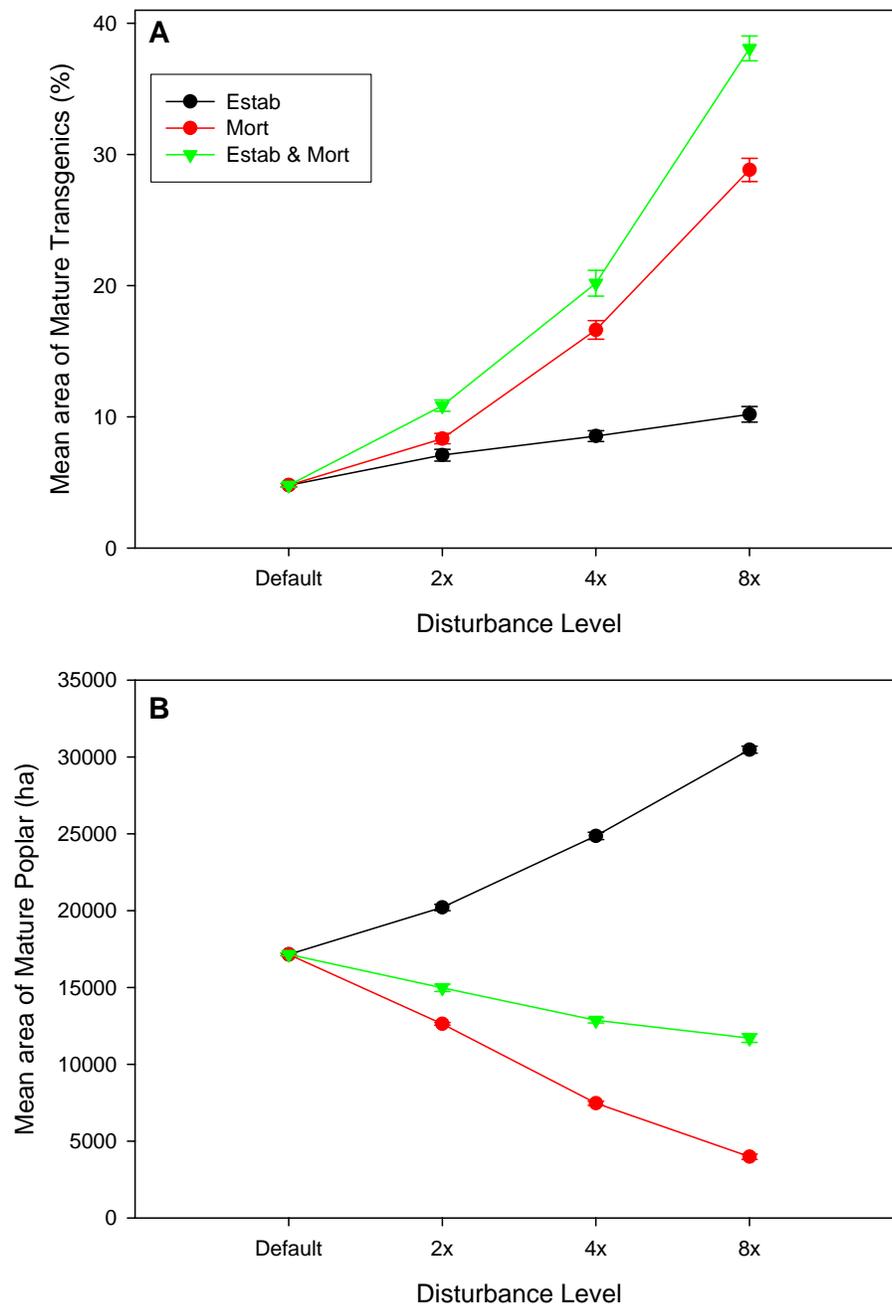
The annual rate of basal area increment had a weak effect on transgene flow. Decreasing the basal area slope by a factor of four resulted in a slight decline in transgene flow, while increasing the slope above the default had little effect (Figures 2.29E and F).

## **Disturbance Regime**

The disturbance regime of the STEVE model is determined by the transition rates from all habitat types to cottonwood (establishment rates), and from cottonwood to all other habitat types (mortality rates). Altering establishment rates had minor effects on transgene flow, while increased mortality rates caused substantial increases in transgene flow (Figure 2.30A). Simultaneously increasing establishment and mortality rates by the same amount caused a similar increase in transgene flow compared to changes in mortality rates alone (Figure 2.30A). Changes that caused large increases in transgene flow also caused a decline in total area of wild poplar (Figure 2.30B).



**Figure 2.29** Effects of poplar characteristics on transgene flow. **A.** Phenology. Phenology values were assigned so that a range of intercompatibility between plantations and wild trees would result. Phenological compatibility is expressed relative to complete compatibility with wild trees (*i.e.*, a single phenology class). **B.** Effect of carrying capacity slope. **C.** Slope of carrying capacity vs. age, which represents maximum number of trees that can occupy a 100 m<sup>2</sup> cell. **D.** Effect of carrying capacity slope on transgenic basal area as a percentage of total wild poplar basal area. **E.** Effect of varying slope of basal area equation. **F.** Basal area versus age with different slopes.



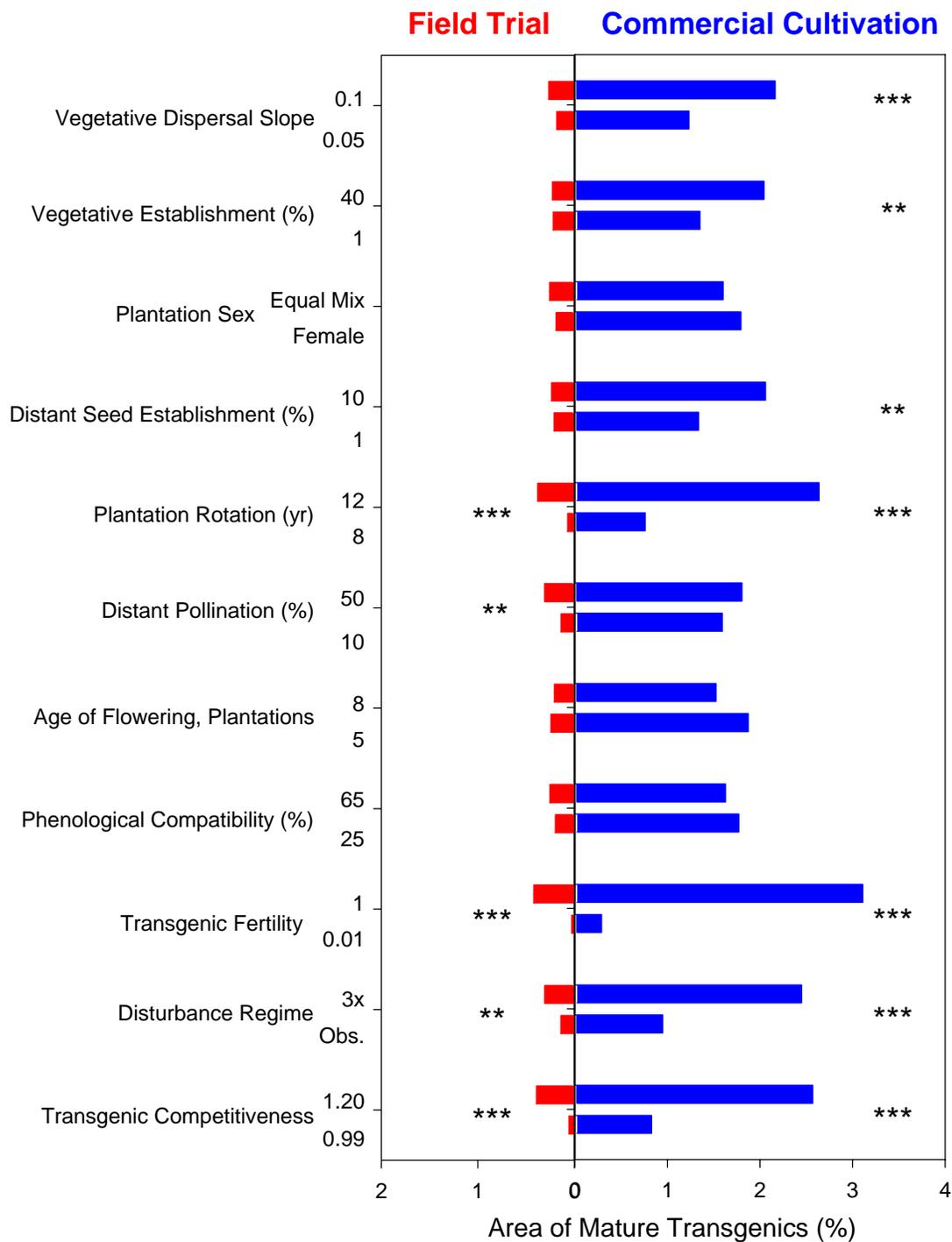
**Figure 2.30** Effect of disturbance on gene flow and poplar populations. **A.** Effects of varying establishment rates, mortality rates, and establishment and mortality simultaneously. Results are presented for rates that are two, four, and eight times greater than the empirical default rates. **B.** Total area of mature wild poplar with each disturbance regime. Enhanced mortality resulted in an overall decrease in wild poplar area, causing enhanced estimates of gene flow (which is expressed relative to total wild poplar area).

## Fractional Factorial Analysis

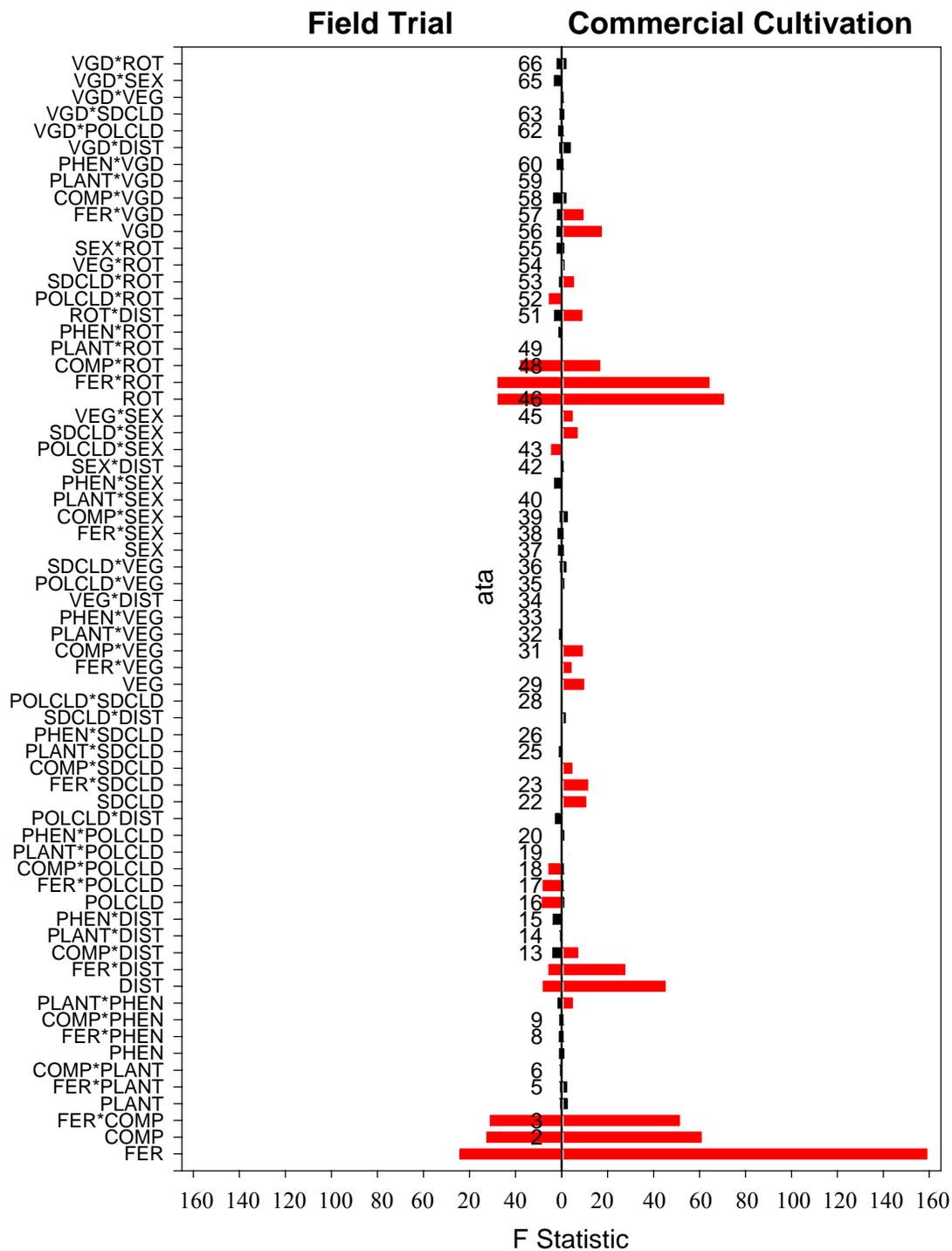
The results for the field trial and commercial cultivation landscapes were concordant for the strongest main effects. Transgenic competitiveness, disturbance regime, transgenic fertility, and plantation rotation all had significant effects on transgene flow for both landscapes (Figures 2.31 and 2.32). Distant seed establishment, vegetative establishment, and slope of vegetative dispersal all had significant effects for the commercial plantations, but not the field trial, though the trends were the same. In contrast, distant pollination was significant for the field trial, but not for the commercial plantations ( $P=0.35$ ), though the trend was again the same (Figure 2.32).

Several interaction terms were also strongly significant for both sets of scenarios (Figure 2.32). Fertility and competitiveness interacted strongly: the effects of a transgenic competitive advantage were largely negated by low fertility (Figure 2.23D). Disturbance and fertility also significantly interacted: the effects of disturbance were disproportionately enhanced under low fertility (Figure 2.33A). Similarly, disturbance had a stronger effect at a lower competitiveness (Figure 2.33B). Rotation length also interacted significantly with both fertility and competitiveness. The effect of increased rotations was strongly diminished by reduced fertility (Figure 2.33C), and rotation was slightly more important with reduced competitiveness (Figure 2.33D). Also, the effect of vegetative dispersal slope was greater with low a level of sexual fertility (Figure 2.33E). Finally, the importance of distant seed establishment was accentuated when all plantations were female (Figure 2.33F).

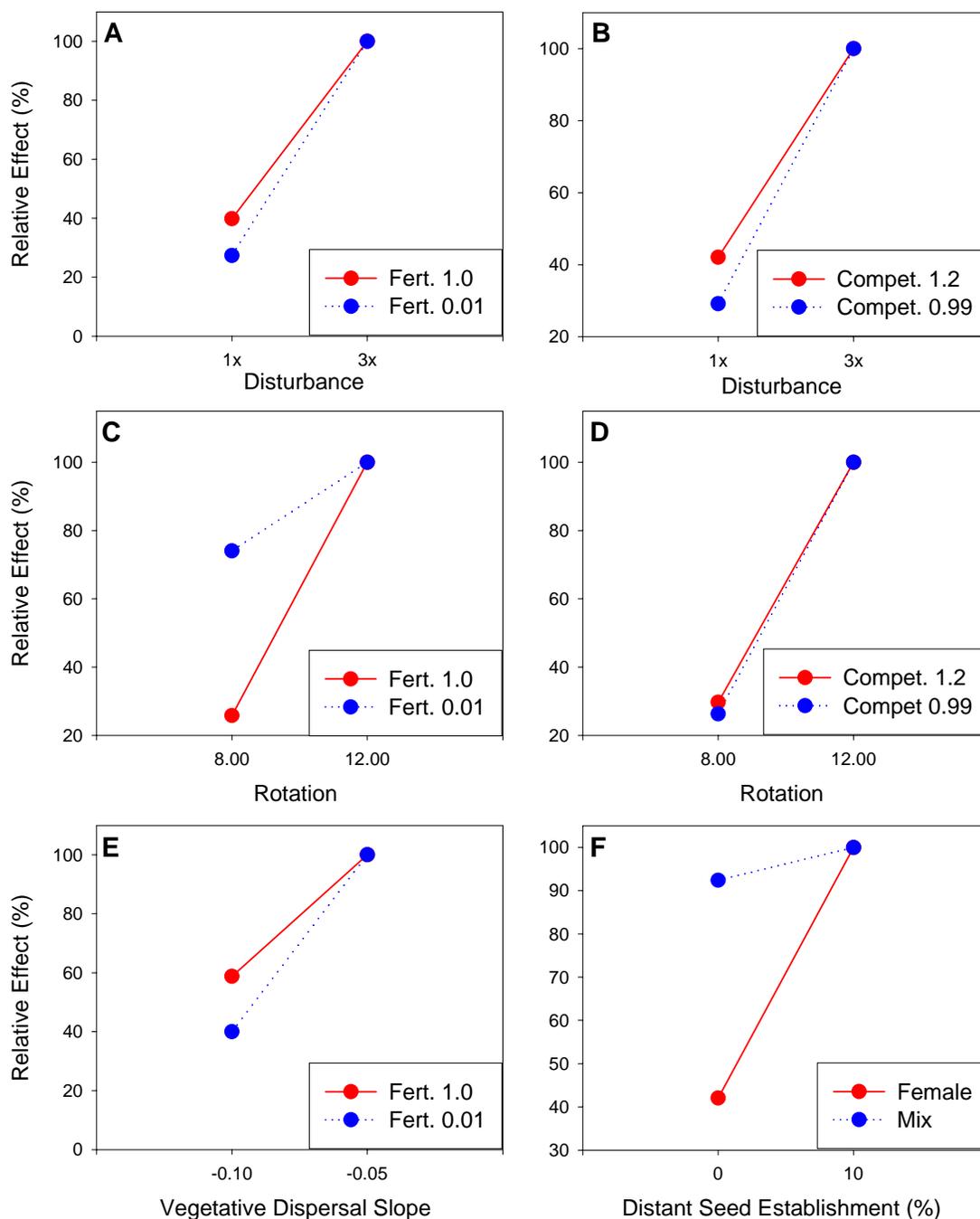
The results of the objective fractional factorial, in which lower and upper values for explanatory variables were set at approximately 20% below and above the default values, were concordant with the other fractional factorial analyses for the strongest effects. Specifically, fertility, transgenic competitiveness, disturbance, and rotation remained strongly significant in the objective fractional factorial. In addition, plantation maturity, phenology, and distant pollination had small but significant effects. Fewer interaction terms were significant in the objective fractional factorial than in the commercial cultivation scenario, but interactions between fertility and competitiveness, fertility and



**Figure 2.31** Least square means for main effects from a resolution V fractional factorial experiment in which 11 main effects and all two-factor interactions were examined. Response was the percentage of area of mature poplar that is occupied by transgenic trees outside of plantations. Results are for a field trial scenario (total plantation area = 19 ha, 100% transgenic) and commercial cultivation (total plantation area = 480 ha, 50% transgenic). See Table 2.9 for information on variables. \*\*\*:  $P < 0.001$ ; \*\*:  $P < 0.01$ .



**Figure 2.32** F-statistics from fractional factorial analysis for a field trial scenario and commercial cultivation scenario. Red bars indicate factors that were significant at  $P < 0.05$ . Variables are defined in Table 2.9.



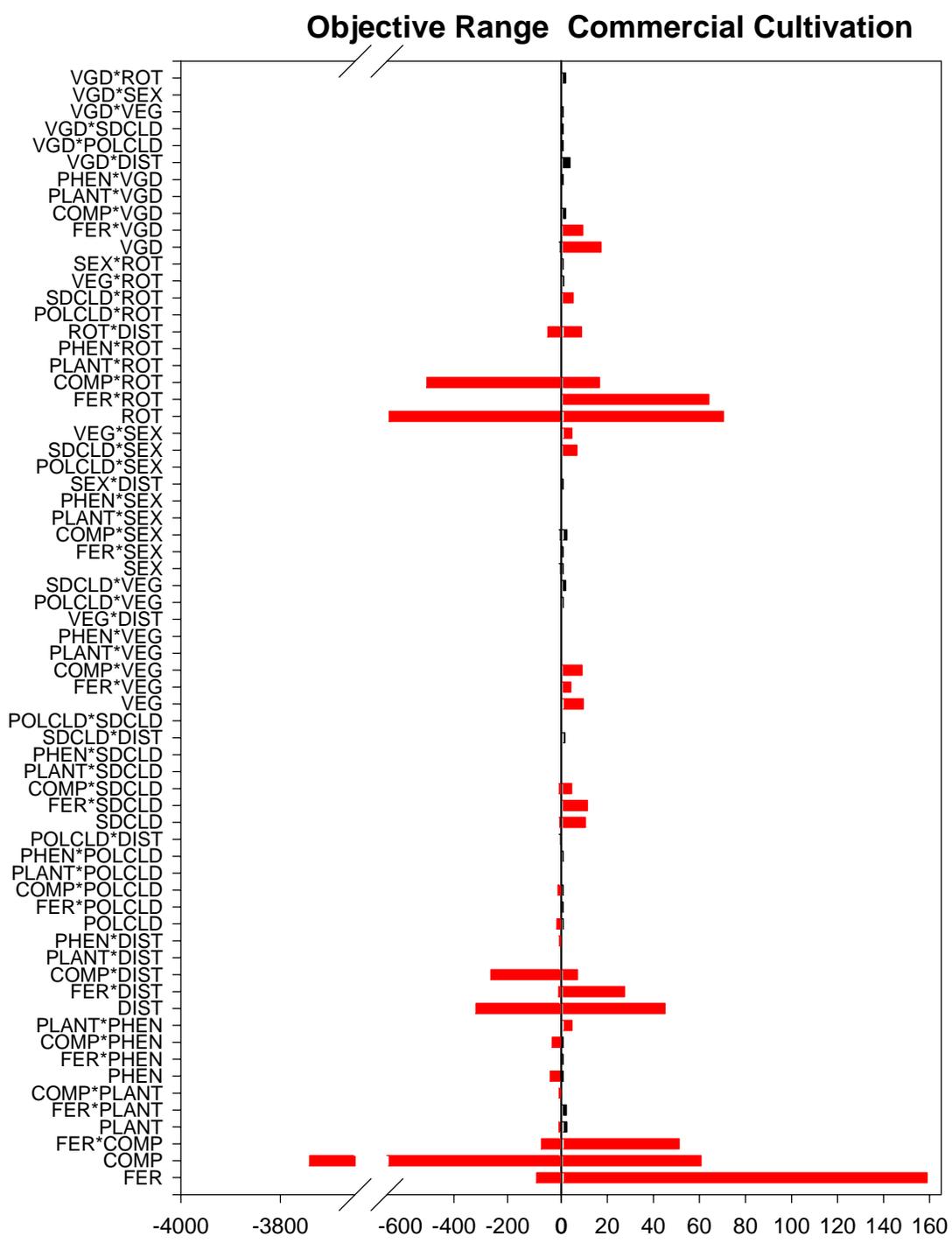
**Figure 2.33** Relative effects of variables in two factor interactions from fractional factorial analysis with commercial cultivation scenario. Relative effects were calculated from least square means at selected factor levels, normalized to maximum value within a factor level. **A.** Relative effect of disturbance at low fertility was a 75% increase in gene flow; at high fertility the effect was just 60%. **B.** Disturbance and transgenic competitiveness. **C.** Plantation rotation and fertility. **D.** Rotation and transgenic competitiveness. **E.** Vegetative establishment and fertility. **F.** Distant pollination and gender of plantations.

disturbance, competitiveness and disturbance, rotation and competitiveness, and rotation and disturbance all remained significant (Figure 2.34).

## **Discussion**

The STEVE model is a formal framework within which the complex processes of transgene flow can be integrated and examined. Development of this model required identification of potentially important processes involved in gene flow, formulation of hypotheses about how these processes should be represented and related, and estimation of the parameters controlling model behavior. This process has systematically probed the levels of knowledge and uncertainty about poplar gene flow on the lower Columbia River.

One of the main findings from the sensitivity analyses is that the model generally responded as expected to alteration in parameter values. This suggests that the underlying logic is generally sound (Haefner 1996; Turner, Gardner, and O'Neill 2001), and that STEVE is a reasonable representation of the system. In addition, sensitivity analyses have revealed parameters that appear to have the strongest influence on gene flow, and those with relatively small effects. Factors with the strongest effects on model outcomes were transgenic competitiveness, transgenic fertility, rates of disturbance, and rotation length of plantations. These factors were all positively associated with transgene flow, as expected. Factors with relatively small effects on gene flow were dispersal distances, relative establishment of seed and vegetative propagules, gender, density, and age of flowering of plantations, overlap in flowering phenology, and wind direction and strength. This information is potentially relevant for setting priorities for future research and monitoring efforts (Dunning, Stewart, Danielson, Noon, Root, Lamberson, and Stevens 1995; James, DiFazio, Brunner, and Strauss 1998; Traynor and Westwood 1999). However, it is important to determine the degree to which findings with the STEVE model are due to flawed model structure or poor parameter estimates, or biologically significant findings relevant to the real world (Conroy et al 1995). We will therefore explore each of the parameters that had particularly strong effects.



**Figure 2.34** Fractional factorial analysis using parameter values varied +/- 20% from our best estimate of baseline values (objective range, Table 2.9) compared to the 'commercial cultivation' scenario (also presented in Figures 2.31 and 2.32). Note broken axis for objective range scenario, necessitated by the extremely high F-statistics for competitiveness.

## Fertility

Transgenic fertility was an extremely important factor controlling transgene spread, even if sterility was far from complete. However, transgene flow was relatively insensitive to reductions below fertility values of approximately 0.1. This finding has great practical significance because engineered sexual sterility is one of the main methods proposed to limit spread of transgenes, but absolute sterility is much more technically challenging than partial sterility (Strauss et al. 1995). As expected, transgenic pollen production and seed production by transgenic females were linearly related to transgenic fertility across the full range of fertility values tested. However, total transgenic seed production and overall transgene flow were both greater than expected under low fertility levels. Vegetative establishment does not account for this discrepancy, because the same pattern was apparent when vegetative establishment was eliminated. At low fertility, the vast majority of transgenic seeds were produced by fully fertile conventional plantation trees pollinated by transgenic plantation males with reduced fertility. This is likely to be a specious result, because the STEVE model allows a proportion of all seeds (50% by default) to be sired by pollen produced in the local neighborhood of 440 m radius. Modeled seed production is not sensitive to the absolute amount of pollen arriving in the female cell, so if transgenic males are the sole pollen source in a neighborhood, half of the seeds produced by a conventional female tree will be transgenic. Seed production for that female will be normal even if all nearby males are nearly sterile. It is reasonable to expect that reproductive success of nearly sterile male trees would be higher in the interior of plantations, where competition from wild pollen is minimized. However, our data on gene flow from nearly sterile triploid male trees do not support this assertion: no pollen flow was detected to females in the interior of the plantation directly adjacent to the male block, despite a lack of fertile male trees in the female's neighborhood (Chapter 3). Therefore, a desirable alteration to the STEVE model would be to allow the proportion of background pollination to be scaled based on the abundance of local viable pollen, though it is unclear how this relationship could be derived.

The effects of reduced fertility were even more attenuated for transgene flow than for seed production (Figure 2.25C). This was probably due to an 'establishment threshold' that is inherent to the STEVE model. Establishment occurs in units of 100 m<sup>2</sup> area, with

a maximum of 2,000 seedlings becoming established in each cell. Establishment of each genotype is proportional to representation in the propagule pool arriving at the cell. Therefore, transgenics must constitute at least 1/2,000 or 0.05% of the propagule pool to become established. Consequently, the model is insensitive to levels of transgenic propagules that fall below this 0.05% threshold. This establishment threshold could be partly ameliorated by incorporating stochasticity into the establishment process, as described below.

The net result of these two idiosyncrasies of the STEVE model is an inadequate representation of transgene flow under very low levels of fertility. On the one hand, gene flow by pollen is overestimated when transgenic fertility is low. On the other hand, establishment by seed is underestimated when transgenic seed production is low. Therefore, the model should be revised before using for detailed explorations of scenarios with nearly sterile transgenics (*i.e.*, fertility of 0.1 or less).

Other investigations have also identified fertility as a major factor limiting plant spread. For example, a reduction of fertility of as little as 75% was projected to limit the spread of scotch broom (*Cytisus scoparius L.*), based on insect-protection assays and simulations (Rees and Paynter 1997). Also, density of pines spreading from plantations in South Africa was sensitive to fecundity and age of reproductive maturity in spatially explicit simulations (Higgins et al 1996). Spread of feral oilseed rape was hypothesized to be limited by seed input based on patterns of establishment along shipping (*i.e.*, dispersal) routes (Crawley and Brown 1995). Finally, a model of transgenic fish introductions implicated fertility as an important factor, potentially offsetting substantial competitive disadvantages in other aspects of the lifecycle such as juvenile survival (Muir and Howard 2001).

Another key finding of the STEVE model was the effect of instability in genetically engineered sterility. Reversion rates had to be relatively high, both in terms of probability (> 1%) and degree (> 10% restoration per reversion event), to have detectable effects on gene flow. Reversion could represent mutations of the transgene, changes in transgene expression, and/or recombination that uncouples sterility from other transgenic traits. Rates of all of these processes would likely be orders of magnitude below the rates required for significant effects on modeled transgene flow (Hartl and Clark 1997). Also,

such high rates of reversion would be readily detected with moderate precommercial screening and post-release monitoring efforts. However, the effects of reversion depend on the details of how reversion to fertility might occur. For example, if modeled fertility restoration was permanent and cumulative (*i.e.*, persisting in subsequent years), the effect on transgene flow was much greater than for transient restoration. Therefore, mechanistic studies of trait stability would be useful.

Stability of gene expression and/or suppression over long time frames and in variable environments is one of the key challenges for transgenic trees in general, (Mullin and Bertrand 1998b), and for biosafety measures like engineered sterility in particular. Because of their large size and long lifespan, individual transgenic trees are subject to a much wider range of temporal variation than individual annual plants, and they would be much more costly to remove if they failed during a rotation. Thus, the standard for genetic stability must necessarily be higher (Ahuja 1997). Stable expression of transgenic traits has been achieved for a variety of species and traits over multiple years and environments, as evidenced by the number of transgenic varieties in production and commercial use (<http://www.isb.vt.edu/CFDOCS/ISBtables.cfm>). However, there are also abundant reports of instability of expression, and transgene silencing is an intensively-studied phenomenon (reviewed by (Finnegan and McElroy 1994; Matzke and Matzke 1998; Stam et al 1997). Experience with stability of gene expression in poplar is relatively limited, but generally shows stable gene expression under vegetative propagation. Expression of the reporter gene GUS (*uidA*) has generally been found to be much more variable under field conditions than in tissue culture or the greenhouse, but stably expressing lines remained stable for four years or more (Pilate et al 1997). Similarly, Strauss and coworkers have observed generally stable transgene expression over several years in the greenhouse and field for vegetatively propagated trees transformed with a variety of genes including GUS (*uidA*), glyphosate resistance (*cp4* and *gox*), glufosinate resistance (*bar*), and BT (*cryIIIa*) (Meilan, Ma, and Strauss 1999; Strauss, Meilan, DiFazio, Brunner, and Carson 2001b). However, stability of transgene expression has never been studied systematically in poplar for a large number of lines under stressful conditions designed to test the robustness of gene expression. Also, there is evidence that transgene expression in poplar becomes more variable in response to

stress (Karnosky et al 1997; Pilate, Ellis, and Hawkins 1997). As data become available on rates and character of reversion to fertility, this information can be incorporated into the STEVE model for explicit simulation of the consequences of expected levels of reversion to fertility.

### **Transgenic Competitiveness**

Transgenic competitiveness had the most dramatic effects on gene flow of any parameter tested. Competitiveness was modeled as growth differences manifested in reduced density-dependent mortality rates for the advantageous genotype, and enhanced density-dependent mortality for the alternate genotype. With this method, a slight advantage results in elimination of the inferior genotype from a cohort, in many cases even when the superior genotype is in the minority. An implicit assumption is that competitive differences are manifested by superior ability to acquire resources, but this does not necessarily imply direct interaction between genotypes. Growth differences could also be due to enhanced tolerance of stress, disease resistance, and/or insect resistance. Therefore, this method of simulating transgenic advantage is flexible and adaptable to a wide variety of traits, as demonstrated by our insect resistance and herbicide resistance simulations (Chapter 4). Another advantage of our approach to simulating transgenic competitiveness is that growth differences are relatively easy to quantify, so model predictions could be readily tested. A disadvantage is that there is no clear relationship with transgenic survival, which depends on starting densities of each genotype, and varies with age. Therefore, the competition differential cannot easily be converted to fitness estimates, and it is difficult to interpret the competition differential in the context of traditional population genetics theory (*e.g.*, Grant 1991). However, the purpose of this model is to study the process of introgression on an ecological (near-term) time scale rather than an evolutionary time scale, so competitiveness is a functional measure of differential success of transgenic trees.

In sensitivity analyses, a competitive advantage of approximately 1.18 or higher resulted in a continual increase in transgenics with no apparent abatement, suggesting that complete introgression in native populations would eventually occur. Competitiveness above approximately 1.25 had little added effect on transgene flow.

This asymptote corresponded to the threshold value at which all newly established poplar cells are destined to become transgenic, because starting densities due to background seed input are above the number required for eventual transgenic dominance of a cohort (Figure 2.20D). Transgenic dominance is assured at all establishment sites at these high transgenic competitiveness values. At this level of competitiveness, gene flow is limited primarily by the availability of establishment sites and mortality rates of native populations, as demonstrated by the extraordinary levels of transgene flow under elevated disturbance and competitiveness.

Our treatment of transgenic competitive advantage in sensitivity analyses was clearly unrealistic because we simulated a constant and uniform advantage over the entire landscape and for the duration of the model runs. The competitive effects of most alleles will vary substantially with environmental conditions (Harper 1977; Hartl and Clark 1997). For example, a transgene that confers drought tolerance will have much stronger effects on relative transgenic competitiveness under xeric conditions than under mesic conditions. Also, the effects of an individual allele occur in the context of the activities of thousands of other loci, potentially representing millions of genotypic combinations, and orders of magnitude more different phenotypes differing along a multidimensional response surface (Wright 1988). The effects of a transgene measured in a small number of hybrid poplar genotypes may therefore be a poor estimate of effects of a transgene introgressed into a diverse wild poplar population. Furthermore, transgenics advantages might well be transient due to a changing environment (biotic or abiotic). For example, anthropogenic global warming is expected to have fairly dramatic impacts on weather patterns over the next several decades (Intergovernmental Panel on Climate Change 2001), and selective pressures on wild poplars will likely shift as well. Also, continued presence of a qualitative disease or insect resistance gene in a population is likely to lead to gradual evolution of resistance in the pest organism (*e.g.*, (Gould 1998; Kareiva 1999), and therefore a substantial reduction in competitive advantage for transgenics over time.

Another major unknown is the average magnitude of competitive advantage that might be expected from transfer of transgenic traits to wild trees. It is of little use to discuss this issue in general, because there is no reason to believe *a priori* that the process of producing a transgenic plant should cause substantially enhanced competitiveness. In

fact, mutations caused by gene transfer and associated *in vitro* culture are likely to reduce wild fitness on average. Fitness effects of transgenes should be considered on a trait-by-trait basis for individual organisms in the context of the environment of release (Miller, I and Gunary 1993; National Research Council 2000; Tiedje, Colwell, Grossman, Hodson, Lenski, Mack, and Regal 1989). For this reason, we have explicitly simulated two prominent traits that are currently under development in poplar: insect resistance and herbicide resistance, and these are discussed in more detail in Chapter 4. However, it is worthwhile to consider in general if it is plausible that an oligogenic change due to insertion of transgenes could produce a 15-25% competitive advantage, which was the level required for continual spread of transgenics in the wild.

Transgenic traits currently under development that could conceivably provide a competitive advantage in the wild include growth enhancement (through changes in plant anatomy, metabolism, and/or nutrient allocation), abiotic stress tolerance (e.g., salinity, cold, heat, drought, heavy metals), and pest resistance (insects, viruses, bacteria, fungi) (Crawley 1990; Mullin and Bertrand 1998a; Regal 1994; Rissler and Mellon 1996; Rogers and Parkes 1995; Tiedje, Colwell, Grossman, Hodson, Lenski, Mack, and Regal 1989). A great deal of effort has been expended to develop transgenic plants in each of these categories, and there have been some notable early successes, with substantial growth improvements under specific conditions attributable to the effects of transgenes (Gallardo et al 1999; Stewart, All, Raymer, and Ramachandran 1997; Tzfira et al 1999). One example, which serves as an illustrative case study, is genetically engineered down-regulation of an enzyme in the pathway for lignin biosynthesis, 4-coumarate:CoA ligase (Pt4CL1) in quaking aspen (*Populus tremuloides* Michx.). Transgenic trees had substantial enhancement of stem, leaf, and root growth in greenhouse (Hu et al 1999) and field trials, with elite transgenic lines growing up to twice as fast as untransformed controls (Jung-Chui Tsai, personal communication). The mechanism for this growth enhancement is unclear, though other aspects of the phenotype, enhanced cell division and elevated auxin metabolism, suggest a role in growth regulation for Pt4CL1 (Hu, Harding, Lung, Popko, Ralph, Stokke, Tsai, and Chiang 1999).

Superficially, such remarkable enhancements in growth and performance of transgenics seem alarming from an ecological standpoint (Mayer 2001; Petrie 2000;

Tømmerås et al 1996), particularly in the light of model results that showed such dramatic effects on transgene spread with only a 25% transgenic advantage. However, data derived from controlled field trials are of questionable utility for predicting transgene effects in wild systems (Bartsch et al 1993; Kareiva et al 1996; Snow and Morán-Palma 1997; Wolfenbarger and Phifer 2000). Transgenic field trials are generally designed to reveal maximum agronomically significant effects of the transgene by minimizing environmental and genetic variation, and creating a selective environment that favors expression of the transgenic advantage. Transgenic field trials usually focus on a small number of elite genotypes, and extreme phenotypes are seized upon and propagated (usually vegetatively for trees). The Pt4CL1 results were obtained for a number of independent transgenic lines derived from a *single* aspen genotype (Hu, Harding, Lung, Popko, Ralph, Stokke, Tsai, and Chiang 1999). Growth is an extremely complex trait, involving coordinate action of thousands of genes, so epistatic interactions with different alleles in a new genetic background could substantially alter the effects of down-regulated Pt4CL1 as introgression into the wild occurs. Furthermore, the field trial environment is qualitatively different from a wild ecosystem. In a field trial there is little to no vegetative competition, topographic and edaphic conditions are quite uniform, resources (light, nutrients, water) are generally plentiful, and non-target herbivores are often controlled or excluded. In contrast, if a transgene is transferred to wild systems, it will be exposed to a full range of environmental variation, and expression could be sensitive to environmental conditions. In particular, silencing of native genes through antisense suppression (the technique used to down-regulate Pt4CL1) can be prone to instability under field conditions, and native gene expression could be restored in unpredictable ways, thereby reversing the transgenic phenotype (Finnegan and McElroy 1994; Jorgensen 1995). However, even if a trait like Pt4CL1 suppression were constant, the enhanced growth phenotype might not be strongly manifested in a wild environment where selective pressures can be drastically different than in a plantation ((Harper 1977; Poppy 2000; Warwick 1991). This is particularly true of a gene like Pt4CL1, which has a large number of well-documented pleiotropic effects. The most ecologically salient of these effects is a drastic reduction in the quantity and alteration in the structure of lignin,

which is integral to support, transport, and defense against pests and pathogens (Dean 2001; Maury et al 1999; Strauss et al 2001a).

Ultimately, the only way to determine the competitive effects of a transgene in wild ecosystems is to perform field experiments in an appropriate range of environments with background genotypes sampled from populations for which transgene introgression is a concern (Crawley et al 2001; Kareiva, Parker, and Pascual 1996; National Research Council 2000; Rissler and Mellon 1996). Data from such field tests could be coupled with information on distribution of selective pressures in the wild (*e.g.*, distribution of soil salinity, temperature, precipitation) and incorporated into a framework such as the STEVE model for explicit simulation of gene flow. In the absence of such data, the STEVE model is of little value for projecting gene flow of particular transgenes. A major barrier to performing such experiments with trees is an extended juvenile period that prevents controlled introgression of transgenic traits into diverse genetic backgrounds by traditional backcrossing (Brunner et al 1998). Fortunately, early flowering varieties of aspen and eucalyptus (*Eucalyptus spp.*) are now available, and these can be initially transformed with a trait of interest and subsequently bred with wild relatives to produce a large number of transgenic genotypes in one to two years (Strauss, Meilan, DiFazio, Brunner, and Carson 2001b). Alternatively, wild relatives could be directly transformed and studied alongside commercially valuable transgenic genotypes.

### **Seed and Pollen Flow**

Parameters controlling the slopes of local pollen and seed dispersal had little effect on transgene flow, primarily because of the spatial distribution of plantations relative to wild populations and establishment sites. Wild females accounted for the majority of transgenic seed production in scenarios with fully fertile transgenics (Table 2.10), yet 61% of transgenic pollinations of wild females originated from the background pollen cloud (not shown). Even more strikingly, 82% of established transgenic seedlings originated from the background seed cloud (*i.e.*, greater than 220 m distance, Figure 2.24C). Thus, most gene flow occurred from outside the pollen and seed neighborhoods, and it is not surprising that the model was relatively insensitive to local dynamics. This

also explains why wind had such a small influence on gene flow: we modeled wind solely as a perturbation of local dispersal probabilities.

Pollen dispersal had a stronger influence on transgene flow than seed dispersal: distant pollination had larger effects on transgene flow than distant seed establishment, and elimination of male plantations caused a reduction in transgene flow, while cultivation of all-male plantations caused an increase in transgene flow. This was partly due to the larger neighborhoods for pollen (440 m) than for seed (220 m). However, another important factor was the continual presence of zones with minimal competition from wild pollen. As explained in the fertility discussion, some conventional female transgenic trees in the interior of plantations had pollen neighborhoods consisting entirely of transgenic males, so 50% of their seeds were transgenic in default scenarios with 50% local pollination. Such situations were extremely rare for seed establishment, because establishment sites rarely occurred simultaneously in close proximity to mature female transgenic plantations and far from wild trees.

Differences between the field trial scenario and commercial cultivation scenario further highlighted discrepancies between pollen and seed dispersal. Distant seed establishment had a significant influence on simulated transgene escape in the commercial cultivation fractional factorial, but not in the field trial fractional factorial. Distant pollination had the opposite pattern: it was significant for the field trial, but not significant for commercial cultivation. The establishment threshold described above is the primary explanation for this pattern. Distant seed establishment was ineffective for the field trial because total transgenic seed production fell below the threshold required for background seed establishment (0.5%). In contrast, the establishment threshold within a seed dispersal neighborhood was much lower (0.0625%)<sup>7</sup>, and transgenic seeds consistently exceeded this threshold in the field trial scenario due to long-distance pollination from the plantation (data not shown). Differences in flowering phenology further favored pollen flow in the field trial scenario. Plantations flowered earlier than

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<sup>7</sup>A maximum of 10% of establishment derived from background seed, and maximum establishment in a 100 m<sup>2</sup> area was 2000 seedlings, so the threshold for transgenics was 0.5% ( $1/((0.1)(2000))$ ) of total seed production on landscape to become established outside seed dispersal neighborhoods of 220 m. In contrast, local seed establishment constituted at least 80% of establishment, so the threshold was 0.0625%, or  $1/((0.8)(2000))$ .

wild trees on average, so there was less competition from wild pollen in pollinating early-flowering wild females, and transgenics constituted a larger proportion of early seed production. We did not model phenological differences in seed establishment, so this temporal refuge from wild competition was not available for seed flow.

The effect of distant pollination was qualitatively the same for the two scenarios, but the effect of distant pollination was weak in comparison to other factors in the commercial cultivation scenario. A possible explanation is that distant seed establishment was sufficient for colonizing long-distance establishment sites in the commercial cultivation scenario, and a reduction in distant pollination had little effect on attenuating gene flow. As described above, distant seed flow results in establishment of some transgenic seedlings at all establishment sites on the landscape, and these might survive long enough to be counted as gene flow, particularly when transgenic trees have a competitive advantage.

Pollen dispersal is generally considered to be more effective for long-distance gene flow than seed, partly because of generally higher dispersal capability due to smaller propagule size, and because pollen gametes are dispersed twice, once as pollen and again as seed (Ellstrand 1992; Wu et al 1998). Our dispersal studies for poplar generally supported this assertion: neighborhoods were about twice as large for pollen as for seed. However, it is important to note that our field studies were designed to detect pollen flow, and our seed flow estimates are approximate due to difficulties in resolving maternity (Marshall et al 1998; Neff et al 2000). Indeed, modeled pollen flow predictions matched observed pollen flow from hybrid plantations much better than modeled seed flow predictions (Chapter 3). Particularly lacking are estimates of long-distance seed dispersal, which could be considerable in poplar. Poplar seeds are quite small, with plumed appendages that facilitate primary dispersal by wind and considerable secondary dispersal by both wind and water (Braatne, Rood, and Heilman 1996; DeBell 1990; Johnson 1994b). The portrayal of seed dispersal in the STEVE model was quite simplified. In particular, we did not simulate directed dispersal along watercourses, which is probably one of the primary means of colonizing new sites for poplars (Braatne, Rood, and Heilman 1996; Johnson 1994a; Noble 1979). Instead, we allowed transgenic establishment in all cohorts, regardless of location, and proportional to total transgenic

and conventional seed production on the whole landscape. This simulates panmixia of propagules, which is probably more realistic for pollen, which can potentially enter the stratosphere (Lanner 1965), than for seed. Therefore, we likely overestimated long-distance seed input for upland locations, and may have underestimated it for areas prone to flooding and deposition of water-dispersed seed.

The importance of long-distance propagule movement is in keeping with current understanding of gene flow dynamics. There are some very obvious cases in which long-distance dispersal is essential for recruitment, such as recolonization of newly emerged or newly razed islands, or intercontinental exchanges of organisms (Sauer 1988). However, there is increasing evidence that long-distance or ‘jump dispersal’ is a critical determinant of spread rates of organisms colonizing new territory (Clark, Fastie, Hurtt, Jackson, Johnson, King, Lewis, Lynch, Pacala, Prentice, Schupp, Webb III, and Wyckoff 1998; Lewis 1997). Early models of spread of invading organisms used a reaction-diffusion approach, which simulates an advancing wave that travels at a constant speed (Kareiva 1990). However, these models vastly underpredicted spread rates in a number of prominent cases, including recolonization by tree species following glacial retreat (Clark, Fastie, Hurtt, Jackson, Johnson, King, Lewis, Lynch, Pacala, Prentice, Schupp, Webb III, and Wyckoff 1998), spread of cereal leaf beetle in North America (Andow et al 1990), and spread of Argentine ants in the southeastern United States (Suarez et al 2001). In all of these cases the rate of spread was orders of magnitude greater than that expected based on a traveling wavefront reaction-diffusion model. The differences are likely due to the importance of disjunct foci formed by rare long distance dispersal events (Clark, Fastie, Hurtt, Jackson, Johnson, King, Lewis, Lynch, Pacala, Prentice, Schupp, Webb III, and Wyckoff 1998). In such cases, the data are best modeled by mixed models that can simultaneously accommodate two separate dispersal mechanisms (Higgins and Richardson 1999)(Shigesada and Kawasaki 1997), which is analogous to the approach we have taken with the STEVE model.

## **Vegetative Dispersal and Establishment**

Dispersal of vegetative propagules sets poplar apart from most other organisms for which transgenic field trials are currently occurring, and it is the potential Achilles heel

of strategies to contain transgene flow by preventing sexual reproduction (Strauss, DiFazio, and Meilan 2001a). Poplar is capable of propagating by several different types of vegetative propagules: whole stems, branches, short shoots, coppice sprouts, and root sprouts (Braatne, Rood, and Heilman 1996; DeBell 1990). Propagation by short shoots in particular (cladogenesis), has been hypothesized as an important mode of reproduction for poplar (Galloway and Worrall 1979). Short shoots abscise at high frequencies annually (Dewit and Reid 1992), can retain viability for extended periods, and can potentially be dispersed considerable distances by water (Galloway and Worrall 1979). There have been numerous studies of clonal structure of poplar populations (Table 2.7), but we know of no direct studies of vegetative dispersal and establishment.

The lack of data on vegetative dispersal and establishment is a gap in the empirical support for the STEVE model. However, modeled transgene flow was relatively unaffected by changes in the slope of local vegetative dispersal and rates of vegetative establishment in sensitivity analyses with fully fertile transgenics. The importance of vegetative dispersal and establishment did increase with decreasing sexual fertility. Moreover, both vegetative establishment and vegetative dispersal were significantly associated with transgene flow in the commercial cultivation fractional factorial analysis. However, the effect was quite weak, and transgene flow with reduced fertility transgenics (0.1 or less) was still an order of magnitude less than for fully fertile transgenics, even with the most liberal estimates of vegetative establishment. This suggests that transgene flow through vegetative propagation will be minor regardless of the parameter values. Nevertheless, a key assumption in the STEVE model is that distant vegetative establishment does not occur at a biologically significant level: that is, the vast majority of vegetative establishment occurs within 220 m of the source tree. This is generally consistent with extant data on clone structure from a diversity of studies (Table 2.7). Major violations of this assumption would result in transgene flow comparable to that observed with female plantations because long-distance movement of vegetative propagules would be similar to long-distance seed movement. Because the model was relatively insensitive to long-distance seed movement, we can still conclude that vegetative dispersal would likely have relatively minor effects on transgene flow, even

with some long-distance dispersal. However, future versions of the model should accommodate long-distance vegetative dispersal so that this may be assessed directly.

## **Plantation Silviculture**

Silvicultural choices may have substantial implications for gene flow by altering the basal area of flowering transgenics on the landscape at a given time. Extended rotations caused particularly large increases in gene flow because mature transgenic basal area increases exponentially with rotation length. Therefore, plantations that are intended for production of solid wood products are likely to have much higher levels of gene flow than plantations intended for pulp production, which have much shorter rotation times (Zsuffa et al 1996). This is likely to become more of a concern in the Pacific Northwest if hybrid poplar growers continue to shift away from fiber production and toward solid wood products. However, current regulations essentially cap rotations at 10-12 years in Washington and Oregon for large poplar farms, because these are the rotation limits for agricultural land use regulations (van Oosten 2000).

Reducing the density of plantations had a weak effect on gene flow, even though this resulted in a proportionate reduction in basal area of transgenics. However, plantation density was varied within a relatively small range compared to other parameters. For example, a reduction from 1,500 trees per ha (tph) to 800 tph resulted in a drop in basal area of 53%, and gene flow levels of around 3%. For comparison, transgenic plantations with 50% fertility had approximately the same level of gene flow as low-density plantations (Figure 2.25A). Poplar plantations are normally between 800 and 2400 tph for most commercial applications in North America, though much lower densities may be used in agroforestry, horticultural, and phytoremediation applications, and in forestry operations in other regions (Zsuffa, Giordano, Pryor, and Stettler 1996).

The size of transgenic plantations also had a large effect on both the quantity and character of gene flow. As expected, gene flow increased nearly linearly with plantation area up to about 10% of the landscape. Theoretically, gene flow should reach a peak at intermediate plantation size and then begin to decline as plantations displace potential establishment sites for transgenics. The dynamics of gene flow differed qualitatively between field-trial plantations (up to 20 ha) and commercial-scale cultivation (*e.g.*, 500

ha). As described above, distant pollen flow was significantly associated with transgene flow for the field trial scenario, but not for commercial cultivation, while seed and vegetative dispersal and establishment showed the opposite pattern. Our estimates of pollen dispersal are much more robust and well-supported than estimates of seed and vegetative establishment, so the gene flow estimates for field trials should be more robust than those for commercial scale cultivation. This suggests that sizeable long-term field trials of fertile transgenic poplars could be conducted with minimal gene flow risk, provided the transgenes do not provide a substantial average selective advantage in the wild. Such trials could provide a means for economically studying long-distance dispersal of seeds and vegetative propagules if efficiently assayed marker genes are included in the transgene construct (Stewart, Jr. 1996; Strauss, DiFazio, and Meilan 2001a).

### **Poplar Characteristics**

Changes in the rate of basal area increment had minor effects on transgene flow across a wide range of values. Basal area primarily affects pollen and seed production in the model, so these changes are of the same magnitude as moderate alterations in fertility. In contrast, altering the slope of carrying capacity resulted in a large increase in transgene flow. This highlights the importance of density-dependent mortality in controlling gene flow. In scenarios with full fertility, transgenics were present in most new cohorts at low frequencies, and were eventually eliminated by density-dependent mortality. However, some of these transient transgenics are counted as ‘gene flow’ because they persist past the age of wild maturity (10 years). Altering the carrying capacity slope had little effect on basal area of transgenics, and therefore probably is not a critical factor for determining potential ecological effects of transgenics.

Flowering phenology was only effective at inhibiting gene flow if there were severe discontinuities between timing of flowering of plantations and wild trees. In fact, minor discontinuities in phenology might enhance gene flow slightly. For example, in western Oregon, peak flowering for hybrids grown in plantations was slightly earlier than for wild trees. This provides a temporal refuge from competition with wild pollen because there will be less competition for pollination of early-flowering females. This is supported by

observations of slightly enhanced transgene flow for simulations with early-flowering plantations compared to simulations with complete flowering synchrony.

An interesting extension of the STEVE model would be to incorporate phenology of seed dispersal and establishment. There is substantial variation in the timing of seed release in wild poplar populations, and reproductive success often hinges on timing seed release with the retreat of flood waters (Braatne, Rood, and Heilman 1996; U.S.Environmental Protection Agency 1999). Patterns of seed dispersal phenology generally parallel those of pollination phenology, with peak seed release for hybrids occurring slightly earlier than the peak for wild trees (personal observation). However, there is a lack of data on timing of disturbance events on the modeled landscape, so it is unclear how dispersal and establishment phenology would affect transgene flow. If there are a substantial number of early establishment events, then dispersal would likely be enhanced because of reduced competitiveness from wild propagules. However, if early establishment opportunities are rare, gene flow would be inhibited for early-flowering transgenics. One may argue that the disjunct phenology of introduced trees is likely to be maladaptive because native trees have been under strong selection to flower and disperse seeds at the most opportune time (U.S.Environmental Protection Agency 1999). However, flood control dams have substantially altered flow regimes in many environments, so evolutionarily selected phenology may no longer be ecologically adaptive (McKay et al 1995; Miller et al 1995; Rood and Mahoney 1990). Therefore, disjunct phenology is not necessarily maladaptive, and in some cases may be strongly advantageous.

## **Disturbance**

Disturbance (creation and destruction of poplar cohorts) by definition is a rate-limiting determinant of transgene flow, setting an upper limit on the amount of establishment and determining the lifespan of cohorts. Accordingly, disturbance rates were consistently a strong determinant of gene flow in sensitivity analyses and all fractional factorial analyses. Disturbance rates had a stronger effect with reduced fertility because the importance of proximity of establishment sites to plantations increased as the representation in the background seed cloud declined. Also, disturbance rates and

proximity of establishment sites were less important when transgenics had enhanced competitiveness, because superior transgenics rapidly dominated a cohort even if under-represented at the time of establishment.

A major effect of enhanced disturbance rates was a decline in area of native poplar populations. This was an enhancement of the trend that was apparent with default establishment and mortality rates derived from a chronosequence of air photos, and is in keeping with trends in other regions where flood control has been imposed (McKay 1996; Miller, Schulz, Hobbs, Wilson, Schrupp, and Baker 1995; Rood and Mahoney 1990). However, our methods for simulating disturbance may lack some important components. For example, a comparison of modeled mortality rates to mortality rates observed in several air photo chronosequences revealed that the modeled landscape experiences a much higher level of turnover through 30 years than was observed for any of the air photo sets. This is partly because multiple transitions to and from poplar occurred in the same locations in the air photos, because some locations are much more dynamic than others (*e.g.*, in-stream islands, (Dykaar and Wigington 2000; Edwards et al 1999; Merigliano 1997). The disturbance algorithm in the STEVE model did not accommodate such complexity, and transitions occurred with equal probability within habitat types, without geomorphic or hydrologic considerations. Also, we only modeled transitions directly to and from poplar because of a lack of data on transition among non-poplar habitat types, and because poplar is a pioneer species. Consequently, large portions of the landscape remained static because they were occupied by habitat types with low rates of direct transition to poplar (*e.g.*, wetland). In reality, significant portions of wetland habitat would convert to palustrine wetland and then to forested wetland and hardwood forest, both of which might include poplar stands (Allen 1999). Therefore, although the general landscape trends were reasonable through 50 years of simulation, some details of spatial location, age distribution, and habitat type are probably inaccurate. Given the crucial role played by disturbance in determining gene flow, it is advisable to improve transition estimates by delineating poplar and other habitat types for several more time points, and to improve the disturbance algorithms to accommodate intermediate transitions.

Disturbance and habitat creation have been found to play a crucial role in other models of tree establishment and recruitment. Reduced fire intervals led to a strong enhancement of modeled pine invasion, though the effect interacted with age to reproductive maturity: if the interval was shorter than time to maturation, invasion was inhibited (Higgins and Richardson 1998). Disturbance (death of adult plants) also interacted with fertility in controlling the spread of introduced *C. scoparius* (Rees and Paynter 1997). Intermediate levels of disturbance have been found to be important in maintenance of species invasion and persistence in a variety of models (*e.g.*, (Lavorel and Chesson 1995; Malanson 1996).

## **Conclusions**

The STEVE model responded as expected to changes in fertility, competitiveness, dispersal, disturbance, and management activities, though some unexpected behaviors emerged as well. Enhanced competitive ability led to a striking increase in transgene flow, especially under enhanced disturbance. Extrapolation of trends suggests transgenes would introgress extensively in native tree populations, with the rate depending on disturbance and the magnitude of the competitive advantage. However, it is unlikely that oligogenic changes produced with current technology could lead to such a strong, uniform, and long-term fitness benefit in the wild. Further research in this area is highly desirable.

The issue of enhanced competitiveness of transgenics may be largely obviated by the use of transgenic trees with greatly reduced fertility. Simulations showed that fertility reductions of 90% or more greatly slowed or prevented the spread of transgenics. However, the structure of the STEVE model causes an overestimation of gene flow at low fertility levels. Therefore, risk assessments for low-fertility transgenics will be conservative in this respect with the current version of the model.

The majority of the modeled gene flow was due to long-distance dispersal, primarily by pollen, though long-distance seed dispersal also played an important role. Much of this establishment was transient for competitively neutral transgenics, because these were mostly eliminated by density-dependent mortality by age 25. Transgenics therefore typically constituted a small proportion of total poplar basal area on the landscape.

Vegetative establishment led to low levels of gene flow for sterile trees, and model outcomes were relatively insensitive to vegetative dispersal parameters. However, additional research is desirable to better-characterize long-distance dispersal of pollen, seed, and vegetative propagules.

Finally, the STEVE model contains a number of implicit hypotheses that deserve empirical testing:

- Poplar transitions most often occur directly to and from the major habitat types delineated on the lower Columbia river. Intermediate transitions are unimportant in overall poplar dynamics.
- Long-distance pollen dispersal results in panmixia of propagules over dozens of kilometers, so there is little spatial differentiation of pollen clouds.
- Long-distance seed dispersal on average constitutes less than 10% of poplar establishment in cohorts.
- Seed clouds are not spatially differentiated over dozens of kilometers.
- Long-distance vegetative dispersal is negligible.

All of these hypotheses have potentially large implications for gene flow, and they warrant further assessment and testing. Confidence in STEVE model results ultimately depends on empirical support for assumptions and parameter estimates. The model must also be validated by comparing empirical estimates of gene flow to model predictions, as described in Chapter 3.

## Chapter 3 Potential Gene Flow from Hybrid Poplar Plantations and Validation of the STEVE Model

### Introduction

A major concern for plant genetic engineering is that introduced genes (transgenes) will be transferred to wild relatives and cause negative impacts in wild and managed systems (Rogers and Parkes 1995; Snow and Morán-Palma 1997; Wolfenbarger and Phifer 2000). This concern is particularly acute for forest trees, many of which are virtually undomesticated and therefore capable of surviving in the wild. Also, many trees have high outcrossing rates, extensive gene flow distances, and are cultivated in close proximity to interfertile wild relatives (James et al 1998). It is therefore especially important to carefully examine gene flow from forest tree plantations, a process that has not been characterized in great detail. There have been a number of studies of 'pollen contamination' and mating within breeding orchards (*e.g.*, (Adams et al 1992; Adams et al 1997; Stewart 1994), and studies of mating patterns within wild populations of forest trees (*e.g.*, (Aldrich and Hamrick 1998; Burczyk et al 1996; Dow and Ashley 1998; Stacy et al 1996), but we know of no studies of gene flow from plantation trees to the wild.

One of the proposed approaches to minimizing undesirable gene flow is the use of lines with low or compromised fertility (Brunner et al 1998; Ellstrand 1988). Clones with putatively low innate fertility are therefore of interest for field testing and early attempts at commercialization, as methods of inducing sterility are under development (Strauss et al 1995). Triploid hybrid cottonwood clones are candidates, because these may have low fertility and desirable commercial traits (Bradshaw and Stettler 1993; Strauss et al 1996). All of the female triploid clones examined by Bradshaw and Stettler (1993) had very low levels of fertility, producing empty seed capsules in controlled crosses. Triploid aspen (*Populus grandidentata* Michaux, *P. tremula* L.) has long been used for commercial purposes (Einspahr 1984), and triploid hybrid cottonwood (*P. trichocarpa* Hooker x *P. deltoides* Marshall) often displays desirable commercial traits (Bradshaw and Stettler 1993).

Here we describe studies designed to help assess the potential for gene flow from hybrid poplar plantations in Oregon. We describe results of controlled crosses involving

triploid clones, as well as field studies of gene flow from large plantations of diploid and triploid trees. We then use results of these studies to assess the performance of a spatial simulation model developed to portray gene flow from transgenic plantations (Chapter 2). The model was originally parameterized with data from gene flow studies within natural populations of black cottonwood (*P. trichocarpa*) (Chapter 2). Therefore, the results reported here are an independent assessment of the performance of the model in portraying seed and pollen flow from plantations.

## **Methods**

### **Controlled Crosses**

Crosses were performed using the “large-slip-in-pot method” in which dormant female branches are rooted in pots and pollinated with pollen collected from forced male branches (Stanton and Villar 1996). The crosses involved six clones, including 4 hybrids (*P. trichocarpa* x *P. deltoides*) and two *P. trichocarpa* trees collected in the wild. The hybrids included a triploid male (24-305), a triploid female (184-402), a diploid male (47-174), and a diploid female (49-177), all of which had been used in commercial-scale cultivation (Table 3.1). The wild trees, PTTG and SF-41, were collected in northern Washington in 1996. The triploids were originally identified based on distinctive foliar characteristics, and later confirmed by flow cytometry (Strauss, Han, James, Brunner, DiFazio, Meilan, and Sheppard 1996).

We collected catkins as capsules began splitting, and counted the total number of seeds produced per catkin. We cleaned seeds using forced air and soil sieves (Stanton and Villar 1996), sowed a portion immediately after cleaning, and stored the remainder at 4° C with desiccant. Fresh seeds were planted in a haphazard design because catkins from each branch matured at different times. To ensure uniform conditions of assessment, seeds from all crosses were sown simultaneously in a second experiment following 30-40 days of storage. We sowed seeds in germination flats containing 5 cm of potting mix (Sunshine #3, SunGro Horticulture, Bellevue, Washington) at 2.5 cm

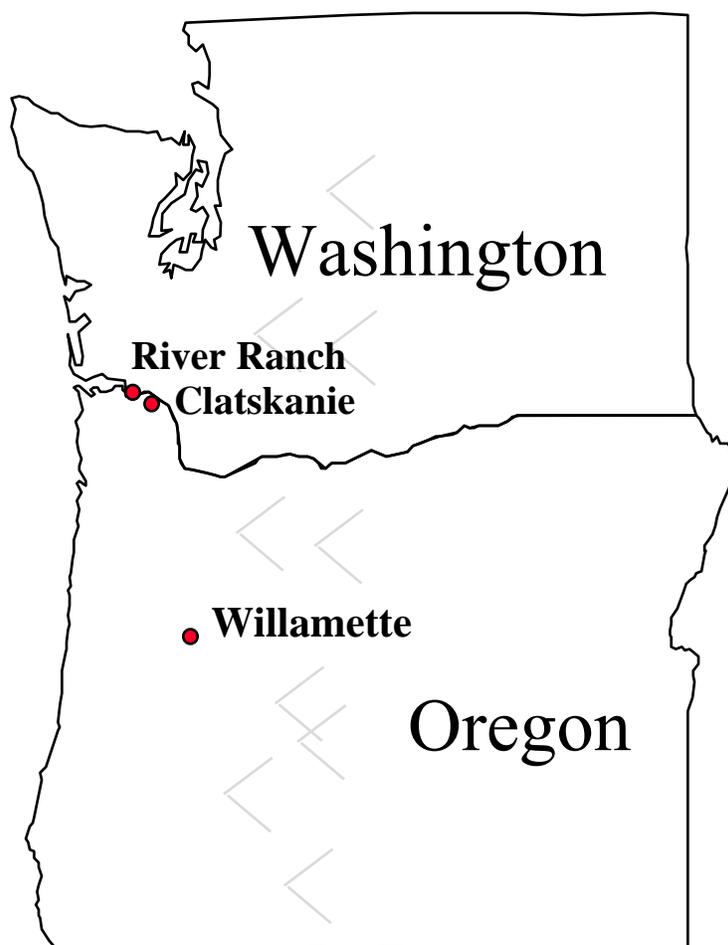
spacing, and maintained seedlings in a greenhouse with daily watering. Height and diameter were measured after 60 days, and survival was measured at 30 days and 3 years.

**Table 3.1** Progeny of controlled crosses for which microsatellite and RAPD genotypes were assessed.

Mother	Father	Progeny
184-402	24-305	29
184-402	47-174	32
184-402	SF-41	33
49-177	24-305	29
49-177	47-174	30
49-177	SF-41	34
PTTG	24-305	20
PTTG	47-174	30
PTTG	SF-41	32

## Field Sites

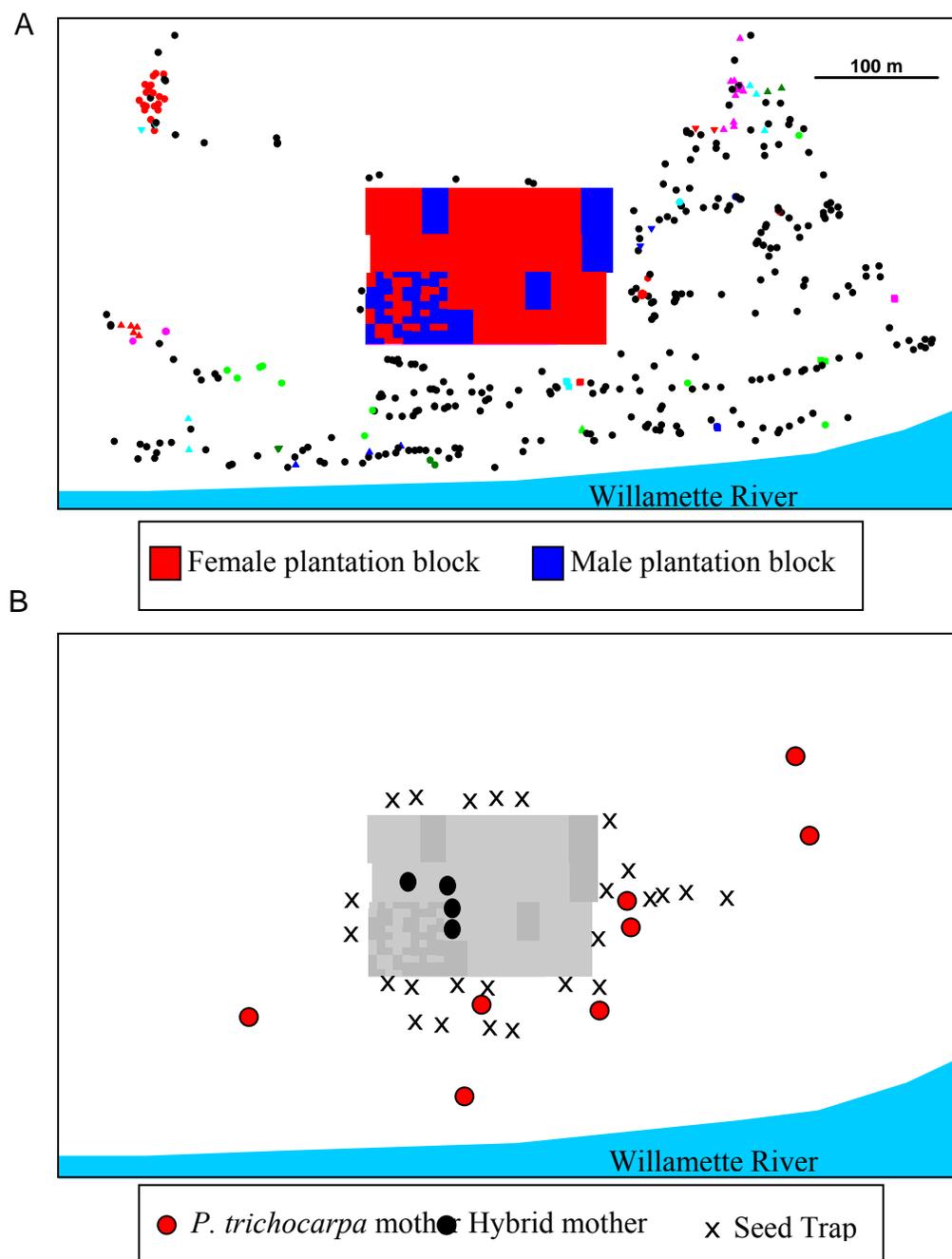
We studied gene flow at three sites in western Oregon with sizeable, flowering plantations of commercial hybrid poplar clones (Figure 3.1; Table 3.2). The Willamette River site is located north of Corvallis, OR, and contained a 2.5 ha plantation that was established in 1990 to test growth of 27 hybrid poplar clones, principally diploid *P. trichocarpa* x *P. deltoides*. Adjacent to the plantation was a riparian population of *P. trichocarpa* consisting of large, mature trees, and an abandoned gravel pit with smaller trees that had apparently become established within the past 20 years (Figure 3.2; unpublished data). The other two sites were large-scale, commercial hybrid poplar farms on the lower Columbia River (Figure 3.1). These sites were chosen because of the presence of large blocks of the triploid hybrid male clone 24-305 and nearby *P. trichocarpa* populations. The River Ranch site was located near the confluence of the Westport Slough and the Columbia River, and contained approximately 110 ha of flowering hybrid poplar clones, including triploid male 24-305, diploid male 46-158, and a variety of additional diploid male clones in small growth trials (Figure 3.3). The Clatskanie site was located near the confluence of the Clatskanie and Columbia Rivers,

**Figure 3.1** Study site locations.**Table 3.2** Study site characteristics.

Site	Elevation (m)	Precipitation (mm)	Degree Days Cooling <sup>1</sup>	Degree Days Warming <sup>2</sup>
Clatskanie/River Ranch	5	1446	2873	944
Willamette	100	1085	2735	1122

<sup>1</sup>Degree days cooling: cumulative number of degrees below 18° C for one year

<sup>2</sup>Degree days warming: cumulative number of degrees above 10° C for one year



**Figure 3.2** Willamette River site. **A.** Locations of plantation trees and wild *P. trichocarpa* trees. Black circles are genets with single ramets. Colored symbols represent genets with multiple ramets. **B.** Locations of *P. trichocarpa* (red circles) and *P. trichocarpa* x *P. deltoides* (black circles) trees from which seeds were sampled, and locations of seed traps.

and contained approximately 20 ha of flowering hybrid poplar clones. Triploid males 24-305 and 23-91, and diploid males 47-174 and 50-194 were present in large plantation blocks (Figure 3.4).

## **Population Sampling**

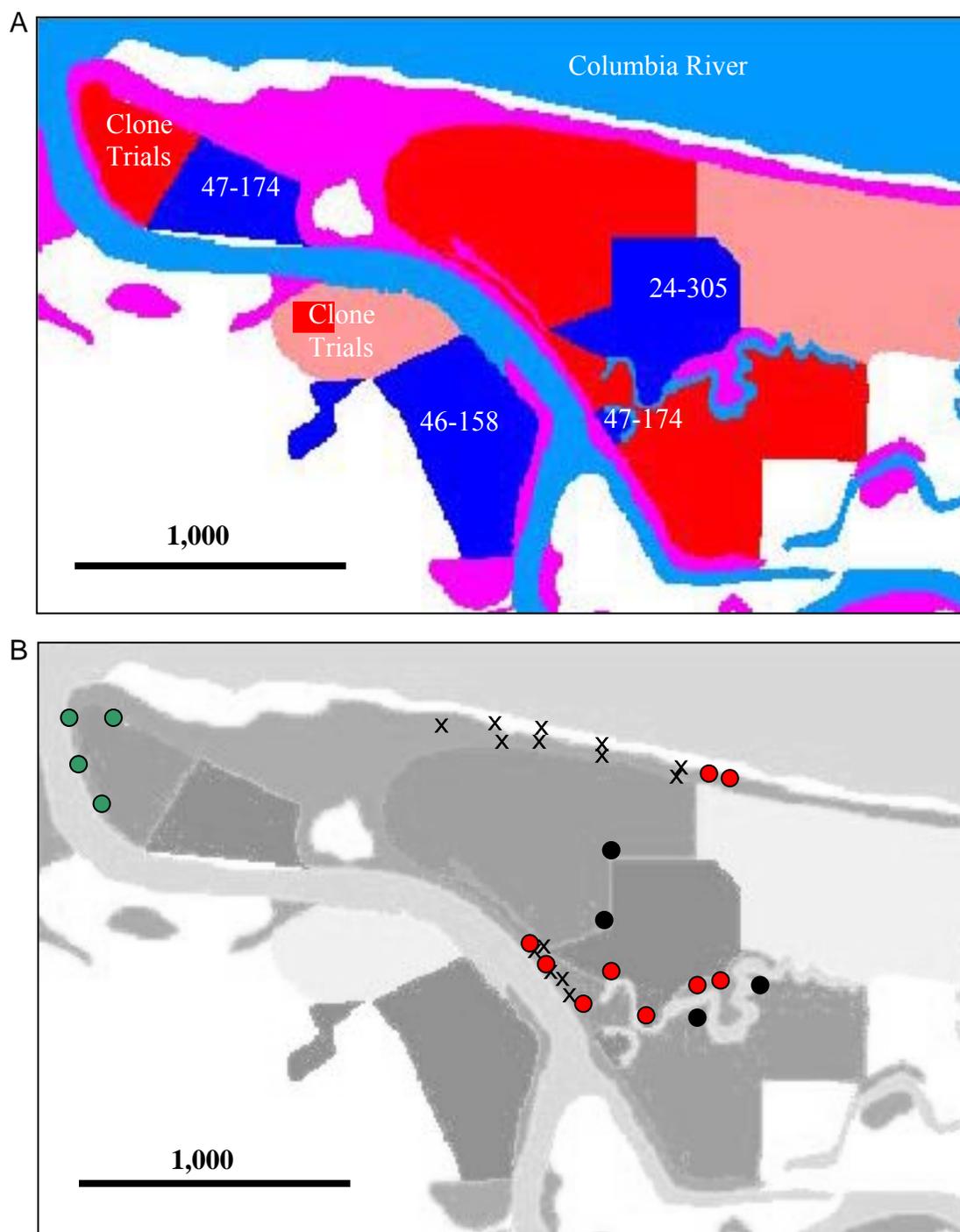
### *Pollen Flow*

We focused on pollen flow from plantation males to wild *P. trichocarpa* females growing in the direct vicinity of the plantations. This was partly because we hypothesized that backcrossing to wild trees would be the primary means of gene flow from plantations because of the extensive potential for pollen flow (Wright 1952), and the importance of backcrossing in enhancing adaptiveness of hybrid progeny in the wild (Snow et al 2001). Also, we sought to maximize the probability of detecting gene flow from plantations because we expected extremely low outcrossing rates based on preliminary controlled cross results. We accomplished this by selecting *P. trichocarpa* trees that were phenologically compatible with plantation males, and growing as close as possible to plantation borders. We also sampled trees in all four cardinal directions around plantations where possible (Figures 3.2, 3.3, and 3.4). Ten to twelve females were sampled per site, and approximately 200 seeds per female were analyzed for hybrid paternity.

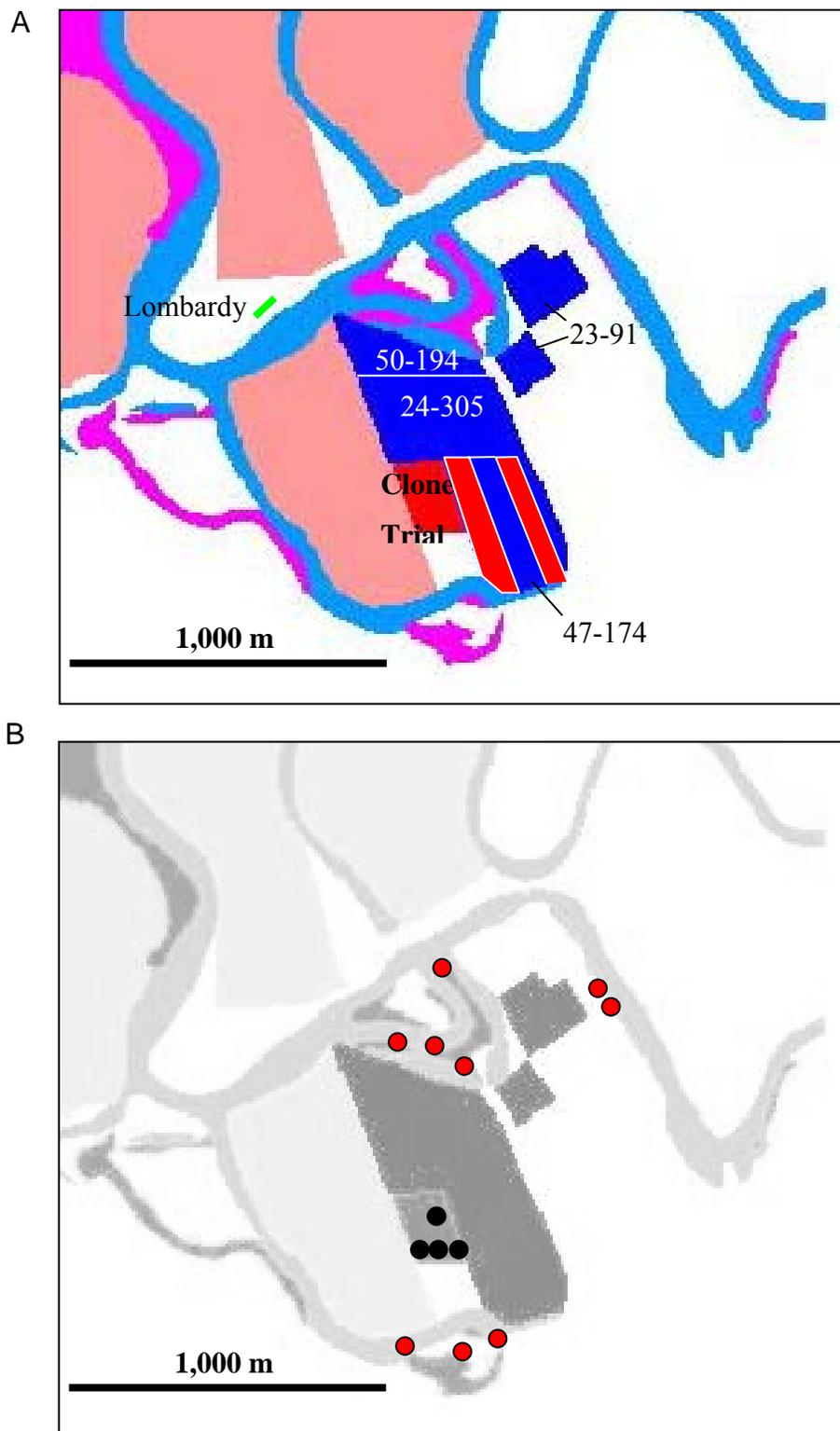
We also collected seeds from hybrid female clones growing within the plantations. However, a comparatively small number of seedlings were analyzed per female because it was not possible to use morphological assays to detect pollination by plantation males, and we had to rely on the more intensive molecular assays (see below).

### *Seed Flow*

We captured seeds in seed traps placed in the vicinity of plantations with flowering female clones (Figures 3.2 and 3.3). Seed traps consisted of nylon mesh bags suspended at the base of vertical screens facing in the 4 cardinal directions. Traps were 0.5 m in diameter and the opening of the bag was 0.5 m above the ground, with 0.5 m of screen



**Figure 3.3** River Ranch site. **A.** Locations of plantation blocks and wild *P. trichocarpa* populations (purple). Pink blocks were immature poplar plantations at the time of the study. **B.** Locations of trees from which seeds were sampled, and locations of seed traps. Green circles represent *P. trichocarpa* females sampled in 1997, red were sampled in 1996. Other colors are same as Figure 3.2.



**Figure 3.4** Clatskanie River site. Symbols are same as Figure 3.3, except lime green indicates location of Lombardy poplar stand.

protruding above the opening. Seeds that hit the screen dropped into the mesh bags, which were emptied twice weekly. Seeds were cleaned and sown fresh, and putative hybrids were subsequently identified (see below).

### *Establishment*

We performed surveys of natural establishment in the vicinity of mature poplar plantations at three sites. One of the sites, Columbia, included both Clatskanie and River Ranch, sites of the pollen and seed flow studies. Hybrid poplar was a relatively recent introduction to this landscape, and large flowering plantations had been present for fewer than ten years at the time of the survey (1996). The other sites were located in Northern Washington (Skagit) and southern British Columbia (Fraser) on unregulated rivers with high rates of disturbance and extensive recent poplar establishment (Figure 3.1). Plantations were also relatively new in the Skagit area, but large flowering blocks of *P. deltoides* x *P. nigra* hybrids had been present at Fraser since the 1960's (van Oosten 2000). We surveyed poplar establishment at all sites by intensively scanning riverbanks and adjacent areas for young trees. We identified putative hybrid progeny based on leaf morphology, and collected tissue for molecular analysis (see below). We also analyzed a random sample of 48 to 96 seedlings at each site using molecular analysis.

### **Identification of Hybrids**

We identified progeny of hybrid parents<sup>8</sup> using a three-tiered approach. We first screened leaf morphology of large numbers of seedlings to identify putative hybrids. However, this method was inadequate for fully characterizing gene flow from plantations because a.) there is a tremendous amount of morphological variation in leaf form of advanced generation hybrids (Campbell et al 1993), so it was unclear if leaf morphology could be effectively used to identify all hybrids; b.) we could not reliably distinguish seedlings derived from plantation trees from those derived from horticultural varieties such as the male Lombardy poplar (*Populus nigra* cv. *Italica* Duroi); and c.) we could

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<sup>8</sup> I use 'hybrid progeny' to refer generically to progeny derived from F<sub>1</sub> plantation hybrids. These progeny may result from first-generation backcrosses to wild *P. trichocarpa* trees (the majority), from intercrosses between F1 hybrids (*i.e.*, F<sub>2</sub>), or from advanced generation backcrosses and intercrosses.

not use leaf morphology to assess pollination of hybrid females by hybrid males. Therefore, we used Random Amplified Polymorphic DNA (RAPD),(Williams et al 1990) analysis to verify the accuracy of morphological designations, and microsatellite (Simple Sequence Repeats, SSR, (Powell et al 1995)) analysis to identify specific parents.

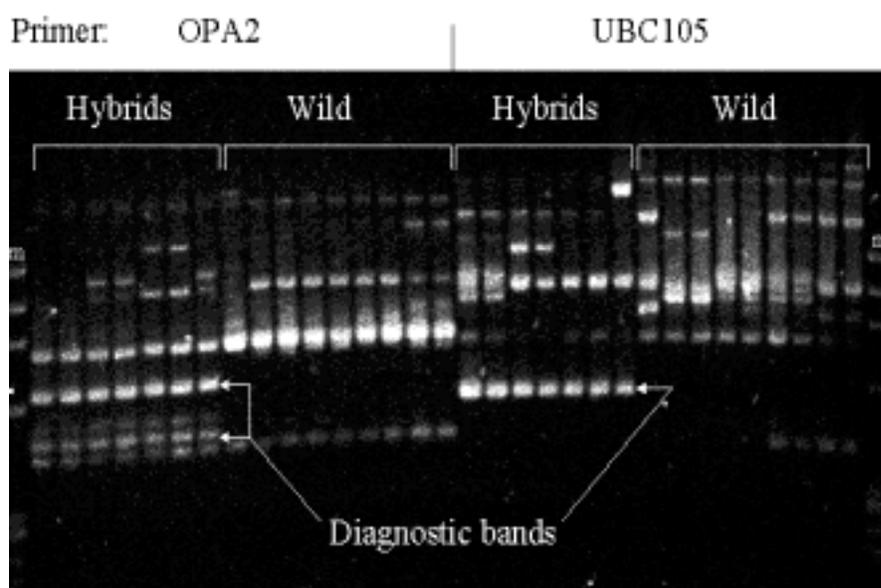
### *Leaf Morphology*

*P. deltooides* does not occur naturally in western Oregon, and there are several morphological characters that distinguish this species from the native *P. trichocarpa* (Eckenwalder 1996). For example, abaxial leaf color and the shape and length of leaf petioles are distinct between the parental species, and hybrid progeny are intermediate. Therefore, we used leaf morphology to screen large numbers of progeny of wild *P. trichocarpa* trees for the presence of hybrid parentage. We assayed leaf morphology in 1-year old seedlings grown first in styroblocks with 2.5 cm spacing (Beaver Plastics Ltd, Edmonton, Alberta) in a greenhouse, and then transplanted to field plots at 15 cm spacing the following spring. This allowed assessment of ‘pre-formed’ leaves (*i.e.*, those derived from overwintering buds), which are more distinctive between sections than ‘new formed’ leaves (Eckenwalder 1996). All morphology assessments were based on the consensus determination of two researchers, and there was greater than 99% agreement between morphological and molecular methods of hybrid identification based on a random sample of 1650 seedlings.

### *Hybrid-specific Markers*

We identified five RAPD loci (Table 3.3) and one microsatellite locus (GWI, Table 3.4) that were present in all 33 *P. trichocarpa* x *P. deltooides* hybrids that were present at the study sites, and absent in a random sample *P. trichocarpa* trees from the same study sites (n=178). These markers were presumably heterozygous in the F1 hybrids, so the probability of correctly identifying hybrid progeny with this method was 0.98 (1-0.5<sup>6</sup>).

We extracted DNA from foliage of seedlings using a protocol developed in our laboratory (available at <http://www.fsl.orst.edu/tgerc/dnaext.htm> ). RAPD amplifications were carried out according to a standard protocol (Aagaard et al 1995) and analyzed on



**Figure 3.5** Agarose gel (2%) showing RAPD bands that are present in hybrids (*P. trichocarpa* x *P. deltoides*) but absent in wild trees (*P. trichocarpa*).

**Table 3.3** RAPD loci used in gene flow studies.

Locus	Primer	Linkage Group <sup>1</sup>
OPA2-640	TGCCGAGCTG	M
OPA2-475	TGCCGAGCTG	A
UBC105-570	CTCGGGTGGG	None
UBC406-700	GCCACCTCCT	None
UBC413-310	GAGGCGGCGA	None
UBC417-1900	GACAGGCCAA	None

<sup>1</sup>Linkage groups determined by mapping on poplar linkage map (Bradshaw et al 1994), by determining genotypes of 90 progeny from family 331. 'None' indicates no significant linkage detected.

agarose gels (Figure 3.5). We included the same three hybrid genets on all gels as positive controls, and repeated reactions for which hybrid-specific bands were not clearly visible.

### *Microsatellite Development and Testing*

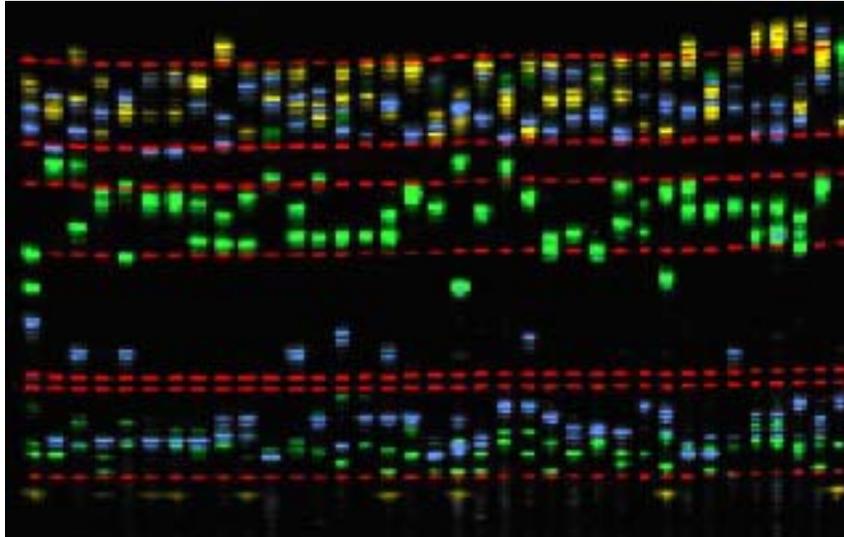
Microsatellite loci were identified from published genomic and cDNA sequences downloaded from GenBank, and sequences derived in-house in a separate project (Brunner et al 2000). In addition, we obtained clones from a genomic library developed at the University of Washington by the Poplar Molecular Genetics Cooperative (PMGC) (<http://www.cfr.washington.edu/pmgc>), and employed microsatellite primers developed by the PMGC. We designed primers using Primer 0.5 software (Whitehead Institute, Cambridge) and amplified microsatellites using the following basic reaction conditions: 6 ng genomic DNA, 1x Gibco BRL (Carlsbad, California) PCR buffer, 1.3 mg/ml bovine serum albumin, 0.1 mM dNTP, 0.25 uM forward and reverse primers, 2-3.5 mM MgCl<sub>2</sub>, and 0.5 units Taq (Gibco BRL). Our basic PCR cycle was:

  
 min.

We optimized each locus for MgCl<sub>2</sub> concentration (between 2 and 3.5 mM) and annealing temperature (between 50 and 56° C).

For initial screening, we spiked amplification reactions with 0.4 uM dCTP or dUTP labeled with a fluorescent dye (Texas Red, Molecular Probes, Eugene, OR; or FAM, HEX, or TAMRA, Applied Biosystems, Foster City, CA). For primers with clear, consistent amplification of a single polymorphic locus, we purchased primers end-labeled with one of the latter three fluorescent dyes, allowing us to multiplex 5 reactions per gel lane (Figure 3.6). We analyzed fragments using the ABI377 automated sequencer (Applied Biosystems, Foster City, CA) with an internal size standard (Genescan ROX 500, Applied Biosystems, or a standard derived from phage PhiX174 and labeled with Texas Red nucleotides ([http://www.fsl.orst.edu/tgerc/Fluor\\_std.htm](http://www.fsl.orst.edu/tgerc/Fluor_std.htm))). We studied 15

loci in total (Table 3.4) and assessed segregation and polysomy (occurrence of more than two alleles in an individual genotype) in approximately 30 progeny for each of the nine controlled crosses (Table 3.1). We also calculated ‘error rates’ for each locus based on lack of maternal alleles in progeny arrays of more than 60 females (unpublished data). The mismatches were due to the presence of null alleles in maternal trees as well as inconsistent amplification and scoring errors.



**Figure 3.6** Image of polyacrylamide gel (6%) showing microsatellite markers. Each lane corresponds to a *P. trichocarpa* individual collected from the Clatskanie population. Loci are PMGC433 (upper yellow), PMGC684 (upper blue), PMGC576 (upper green), PMGC2235 (lower blue), and PMGC420 (lower green).

### Parentage Analysis

We selected the 10 most polymorphic and consistently amplified loci for use in paternity analyses (Table 3.5). We devised a program (Micropat, available from the author) to perform paternity and maternity exclusion analyses (*e.g.*, (Chakraborty et al 1988; Meagher 1986). Micropat functions with raw allele sizes or binned alleles, and can tolerate mismatches caused by null alleles. However, error rates for our loci were considerable (Table 3.5), so methods relying on pure genotypic exclusion of putative fathers would result in a high type I error rate due to erroneous exclusion of true fathers (Pemberton et al 1995). An alternative approach that allows for mismatches due to genotyping errors is the ‘most likely’ method, as implemented in the Cervus program

**Table 3.4** Microsatellite loci used in analyzing controlled crosses.

Locus Name	Source <sup>1</sup>	Repeat Motif	Linkage Group <sup>2</sup>	Forward Primer	Reverse Primer
AG1	Brun	AT	Q	CTTGTAATTAAGAGCAAGCCA	ATGTTAAACTACCTCAAACATATCC
AG2	Brun	AT	E	CGAATATAGTGGATGGTTATTG	CGAATCTGAGTAGGAGAGATG
GWI <sup>3</sup>	Gbnk	AT	D	AAAGGAAAGAGATGTTGG	TAATTTGAGTATGTGAGCTTT
WOU	Gbnk	AG	J	GAGATATTCAAGAGAAATACGTTTAG	ACATGCGCGACATGAG
PMGC14	PMGC	CTT	None	TTCAGAATGTGCATGATGG	GTGATGATCTCACCGTTTG
PMGC204	TGERC	CTT	None	CGAAGATAAATTCTGCAGCTC	TAACCTTCCCCGCATGT
PMGC325	PMGC	CTT	None	CGATTTATGACAGACAGCTTG	GTACCGTTGAGGTGGCTAG
PMGC420	TGERC	AG	X	ATGGATGAGAAATGCTTGTG	ACTGGCACACTCTTTAACTGG
PMGC433	TGERC	AG	D?	GCAGCATTGTAGAATAATAAAAG	AAGGGGTCTATTATCCACG
PMGC576	PMGC	AG	B	GCTGCTTAACATGCCATTGC	AATTTACATTTCTTTATCATCACC
PMGC603	PMGC	AG	None	GTACCTATGAAAGTAGGCAACAC	TTTTTTATCACTATCTCAGATAC
PMGC684	PMGC	AG	M	GAAATTGAATATCTCTCACTTACC	TAATACGTGAAAAGTCAGGTTTTG
PMGC2011	PMGC	AG	E	TCTACGAGGAAAGGGAAGGG	CTTTATAATGCATCATAAAGTTCC
PMGC2156	PMGC	AG	B	GATCTCTCTTACATCACTCATC	GAATGTCTTTACTCCATTGTTGG
PMGC2235	PMGC	AG	I	GCCAAAATAGTAAGTGTGATGG	CACACATTCTCTCATTCAAAGC

<sup>1</sup>Brun, (Brunner, Rottmann, Sheppard, Krutovskii, DiFazio, Leonardi, and Strauss 2000); Gbnk, sequences downloaded, and primers designed in house; TGERC, genomic DNA cloned by PMGC, subsequent sequencing, primer design, and testing done in house; PMGC, primers developed by Poplar Molecular Genetics Cooperative at the University of Washington

<sup>2</sup>Linkage group determined as described in Table 3.3 footnote. Question mark indicates low confidence (LOD < 3).

<sup>3</sup>GWI amplifies the gene *win3*, and is useful for distinguishing *P. deltoides* from *P. nigra* and *P. trichocarpa* (Heinze 1997).

(Marshall et al 1998). However, the ‘most likely’ method is prone to Type II errors in paternity assignment, because homozygous individuals have inherently higher Mendelian transition probabilities than heterozygous individuals (Devlin et al 1988), so highly homozygous males with multiple mismatched loci can still have substantially higher transition probabilities than the true father. We therefore used both Cervus and Micropat in our parentage analyses. For each seedling we examined the two most likely parents by Cervus criteria, as well as all males that matched perfectly by Micropat criteria, and manually excluded putative parents based on credible mismatches (*i.e.*, those unlikely to result from null alleles, failed amplification, or scoring errors). Final parentage assignments were corroborated by RAPD and morphological designations where possible.

**Table 3.5** Loci used in paternity analyses. Data are for adults from the Willamette population, including 27 hybrid clones from the plantation.

Marker	Number of genets	Number of Alleles	Expected Heterozygosity	Exclusion Probability <sup>1</sup>	Error Rate <sup>2</sup>
AG1	227	30	0.8708	0.756	0.109
P14	263	12	0.7783	0.5808	0.003
P2011	228	19	0.8681	0.7421	0.055
P2156	242	21	0.8527	0.7103	0.057
P2235	261	27	0.9236	0.8463	0.146
P420	263	19	0.8768	0.7528	0.039
P433	249	17	0.9029	0.8065	0.046
P576	264	38	0.917	0.8352	0.03
P684	261	23	0.9082	0.8158	0.071
WOU	263	9	0.1102	0.0581	0.002

<sup>1</sup>Exclusion probability is the probability that two randomly sampled, unrelated males in the population will produce gametes with different genotypes (Chakraborty, Meagher, and Smouse 1988).

<sup>2</sup>Error rate is the proportion of known progeny that did not possess a maternal allele, based on 61 to 74 families per locus.

## **Steve Model**

We integrated gene flow data into the Simulation of Transgene Effects in a Variable Environment (STEVE) model, a spatially explicit representation of poplar gene flow (Chapter 2). The model operates on a landscape grid containing information about elevation, habitat type, and poplar populations. The simulation has an annual time step, with modules to simulate creation and conversion of poplar patches, growth, reproduction, dispersal, and competition in poplar cohorts. The simulations track two genotypes, transgenic and conventional. Transgenic trees originate in plantations and may spread to the wild through pollen, seed, and/or vegetative propagules. Propagules are produced in each location proportional to basal area of each genotype, modulated by a fecundity factor. Dispersal occurs explicitly within local ‘neighborhoods,’ and pollen and seed are also distributed across the entire landscape at a low frequency.

## **Model Validation**

One of the purposes of the STEVE model is to provide information on potential gene flow from transgenic plantations that might be useful for informing regulatory decisions and designing monitoring programs for transgenic trees. Therefore it is important to ensure that absolute estimates of gene flow are in the range of empirical observations. We used the STEVE model to generate gene flow predictions for comparison with empirical gene flow estimates. We first derived spatial databases representing each of the three sites used in the studies of hybrid pollen and seed dispersal, treating hybrid plantations as though they contained a selectively neutral transgene marker. We then performed 10 iterations of the STEVE model and tallied the proportion of plantation-derived seeds (hereafter called transgenic) produced by wild trees in 100 m distance classes, and proportion of transgenic seeds in establishment sites at various distances from the edges of plantations. We repeated the analysis for three levels of transgenic fertility, assuming that extant hybrids would have lower effective fertility than wild trees, as illustrated by the controlled crosses. Finally, we compared the simulated data to pollen and seed dispersal curves generated by the parentage analyses described

above. We used the minimum distance from female *P. trichocarpa* trees to mature plantations for pollen dispersal curves, and the minimum distance from seed traps to plantation edges for seed dispersal curves.

## **Results**

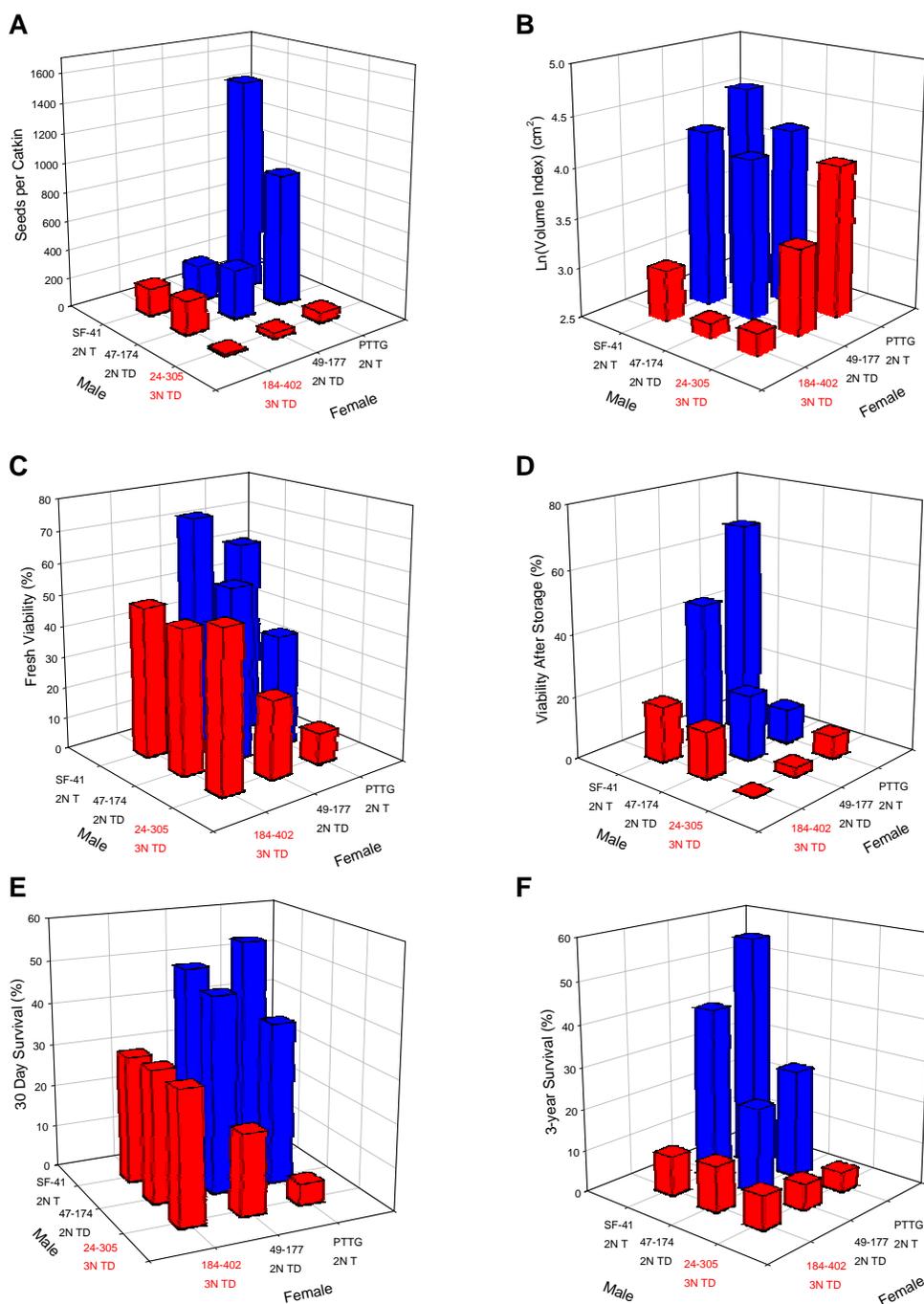
### **Controlled Crosses**

Controlled crosses involving triploids resulted in substantially lower seed production per catkin than crosses involving diploids, and hybrid parents yielded fewer seeds than crosses between *P. trichocarpa* trees (Figure 3.7A). In addition, growth, seed viability, retention of viability, and survival were all compromised in progeny of triploid trees, and progeny of the cross involving two *P. trichocarpa* parents generally performed best (Figures 3.7B-F). Progeny of triploid trees also had much higher rates of polysomy (loci with 3 or more alleles for a seedling) than progeny of diploids (Figure 3.8A), and seedling volume was negatively correlated with degree of polysomy (Figure 3.8B).

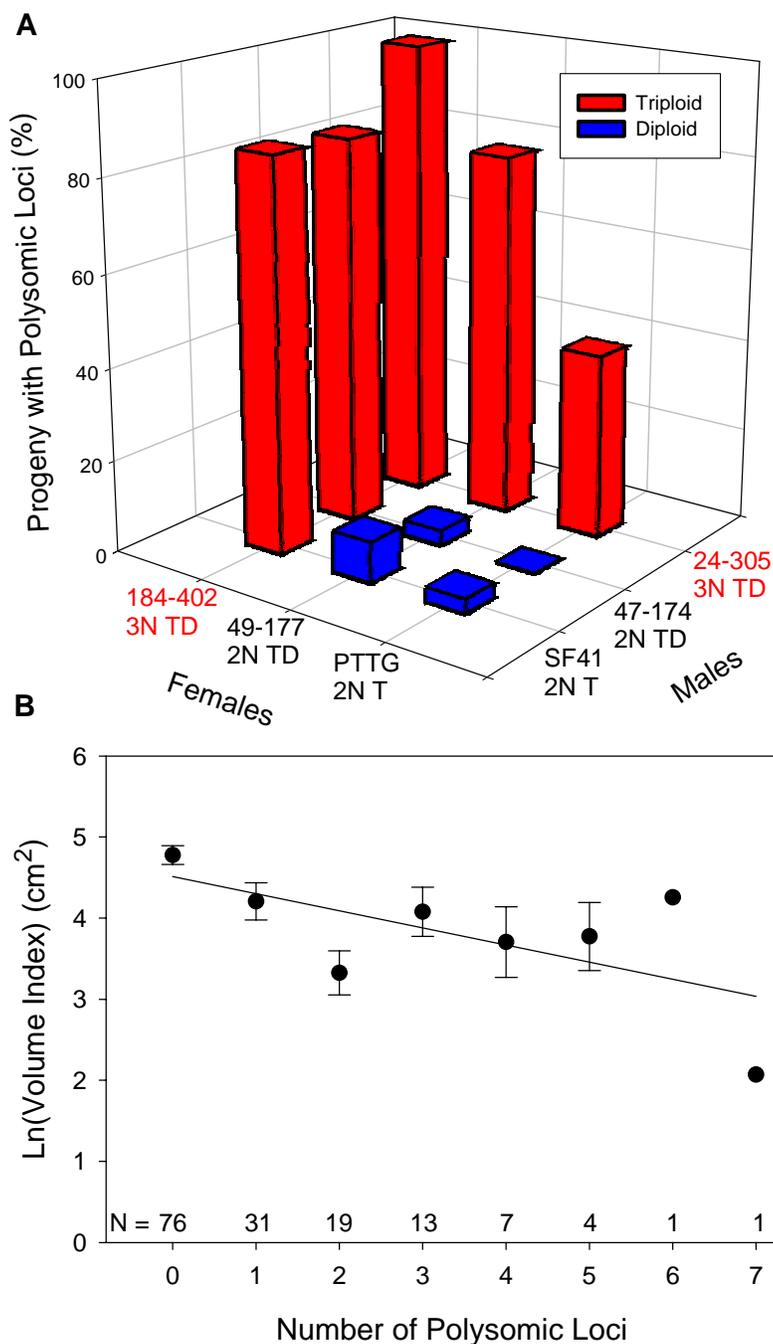
### **Pollen Flow from Plantations**

Hybrid male trees accounted for less than 1% of seeds produced by sampled *P. trichocarpa* trees at all sites (Figure 3.9A). Pollination success was uniformly low for all hybrid clones, and we failed to detect paternity for the majority (Tables 3.6, 3.7 and 3.8). We detected no pollinations for the triploid clone 24-305 at the Willamette and Clatskanie sites (Tables 3.6 and 3.8), but there were 3 pollinations for this clone at River Ranch (0.18% of 1679 seeds analyzed) (Table 3.8). The highest pollination success for a hybrid was for clone 50-194, which pollinated 0.2% of 2266 seeds examined at Clatskanie in 1997 (Table 3.7). Lombardy poplar had comparable levels of reproductive success at all sites, despite being absent from two of the three sampled areas (Figure 3.9A, Tables 3.6 and 3.7).

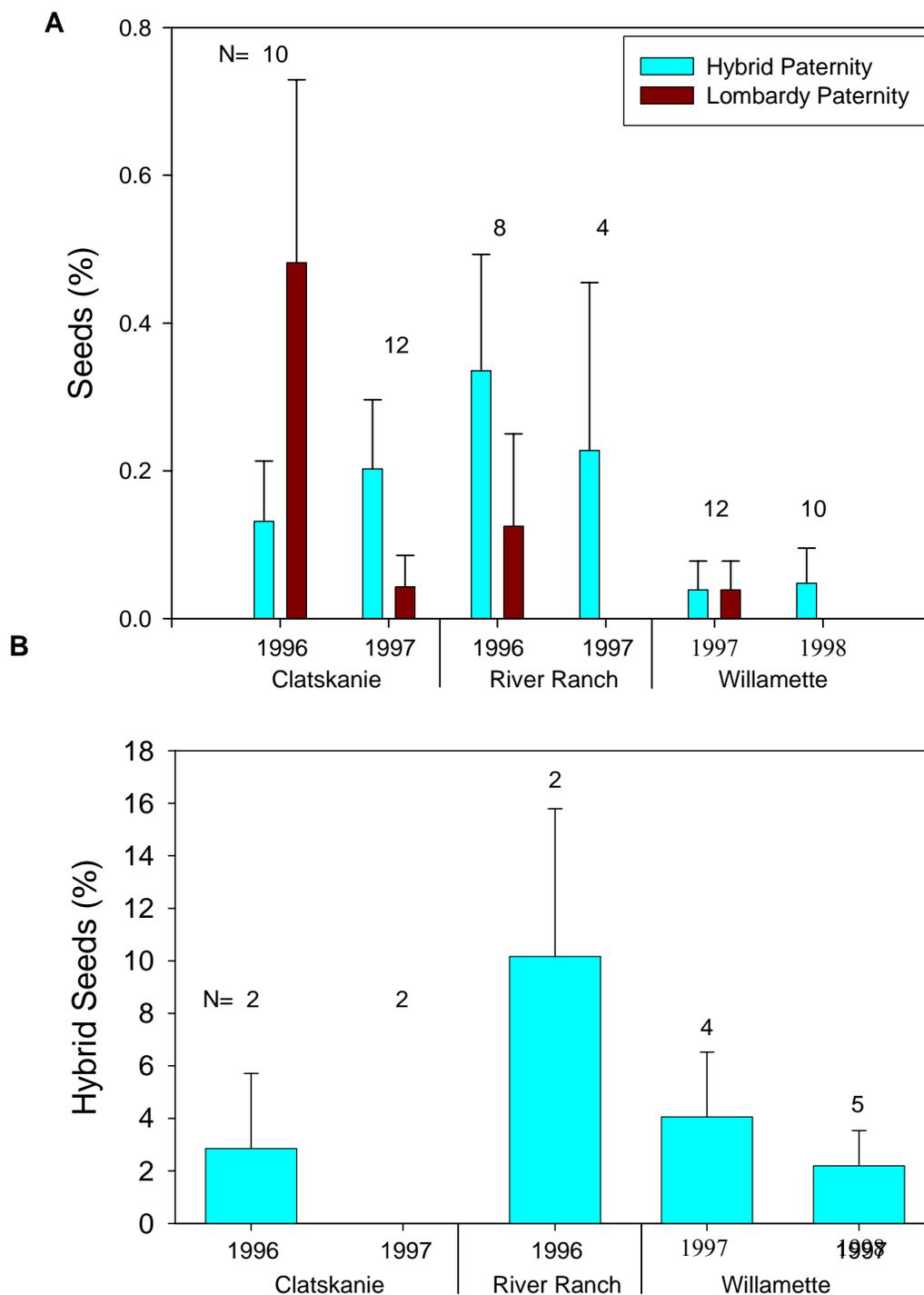
Hybrid male reproductive success was apparently higher with hybrid females in the same plantation than with wild females. Hybrid males pollinated at least 2% of hybrid female seeds in four of five situations examined, with rates up to 10% at River Ranch



**Figure 3.7** Results from controlled crosses involving triploid (3N, red) and diploid (2N, blue) *P. trichocarpa* x *P. deltoides* (TD) hybrids and *P. trichocarpa* (T) wild trees. **A.** Seed production per catkin. **B.** Volume index (height)(diameter)<sup>2</sup> after 60 days of growth in greenhouse. **C.** Germination rate for fresh seeds. **D.** Germination after 30 days of storage at 4° C. **E.** Survival after 30 days. **F.** Survival after 3 years.



**Figure 3.8** Polysomy and growth of progeny of controlled crosses, based on analyses of 15 microsatellite loci. **A.** Occurrence of polysomy by cross. **B.** Volume index after 6-days of growth in a greenhouse, versus number of polysomic loci. Number of progeny observed in each class is indicated above x-axis. Equation and line are from least-squares regression. Bars represent standard errors.



**Figure 3.9** Pollen flow from hybrid poplar plantations and Lombardy poplar. **A.** Paternity of seeds collected from wild *P. trichocarpa* trees near plantations. Bars represent standard errors among mothers, and numbers above error bars are number of mother clones. **B.** Hybrid paternity among seeds collected from female hybrid trees in plantations. There were no progeny of Lombardy poplar among these seedlings.

**Table 3.6** Reproductive success of male hybrid clones with plantation hybrid or wild *P. trichocarpa* females at Willamette River site.

Clone	Area (ha)	1997		1998	
		Wild Mother	Hybrid Mother	Wild Mother	Hybrid Mother
50-179	0.3	0	0	1	0
11-11	0.02	0	0	0	0
11-5	0.02	0	0	0	0
184-401	0.02	0	2	0	0
184-411	0.02	0	1	0	0
194-518	0.02	1	0	0	0
194-527	0.02	0	0	0	0
194-574	0.02	0	0	0	0
196-553	0.02	0	0	0	0
23-96	0.02	0	0	0	0
24-128	0.02	0	0	0	0
24-305	0.02	0	0	0	0
271-88	0.02	0	0	0	0
281-175	0.02	0	0	0	0
47-174	0.02	0	0	0	0
50-182	0.02	0	0	0	0
50-194	0.02	0	0	1	0
52-237	0.02	0	0	0	0
Lombardy	Not present	1	0	0	0
Total Seeds Analyzed	---	2534	71	2120	98

**Table 3.7** Reproductive success of male hybrid clones with plantation hybrid or wild *P. trichocarpa* females at Clatskanie River site.

Clone	Area (ha)	1996		1997	
		Wild Mother	Hybrid Mother	Wild Mother	Hybrid Mother
23-91	3	1	0	0	0
24-305	8	0	0	0	0
47-174	3	0	4	0	0
50-194	2	2	0	5	0
Lombardy	0.02	7	0	1	0
Total Seeds Analyzed	--	2266	125	2489	64

**Table 3.8** Reproductive success of male hybrid clones with plantation hybrid or wild *P. trichocarpa* females at River Ranch site.

Clone	Area (ha)	1996		1997 <sup>1</sup>
		Wild Mother	Hybrid Mother	Wild Mother
24-305M	7.6	3	0	0
46-158M	7.2	0	0	0
47-174M	5.6	0	6	0
11-11	0.016	0	1	0
11-5	0.016	0	0	0
184-411	0.008	0	1	0
189-427	0.008	0	0	0
194-518	0.008	0	1	0
196-553	0.024	0	0	0
196-559	0.008	0	0	1
199-574	0.024	0	0	0
200-596	0.008	0	0	0
20-66	0.01	0	0	0
21-82	0.01	1	0	0
23-91	0.01	0	0	0
24-112	0.01	0	0	0
44-136	0.01	0	0	0
44-150	0.01	0	0	0
50-179	0.016	1	0	0
50-184	0.01	0	0	0
50-194	0.016	0	0	1
55-266	0.01	0	0	0
84-297	0.01	0	0	0
Lombardy	Not present	1	0	0
Total Seeds Analyzed	--	1679	60	880

<sup>1</sup> A different group of wild trees was sampled in 1997 because part of the plantation was harvested (Figure 3.3).

(Figure 3.9B), though no hybrid paternity was detected among 60 progeny of hybrid females in 1998 at the Willamette site (Figure 3.9B). Clone 47-174 accounted for the majority of this hybrid to hybrid gene flow at Clatskanie and River Ranch, though five other male hybrids also bred successfully with hybrid females (Tables 3.6,3.7, and 3.8).

## Model Validation

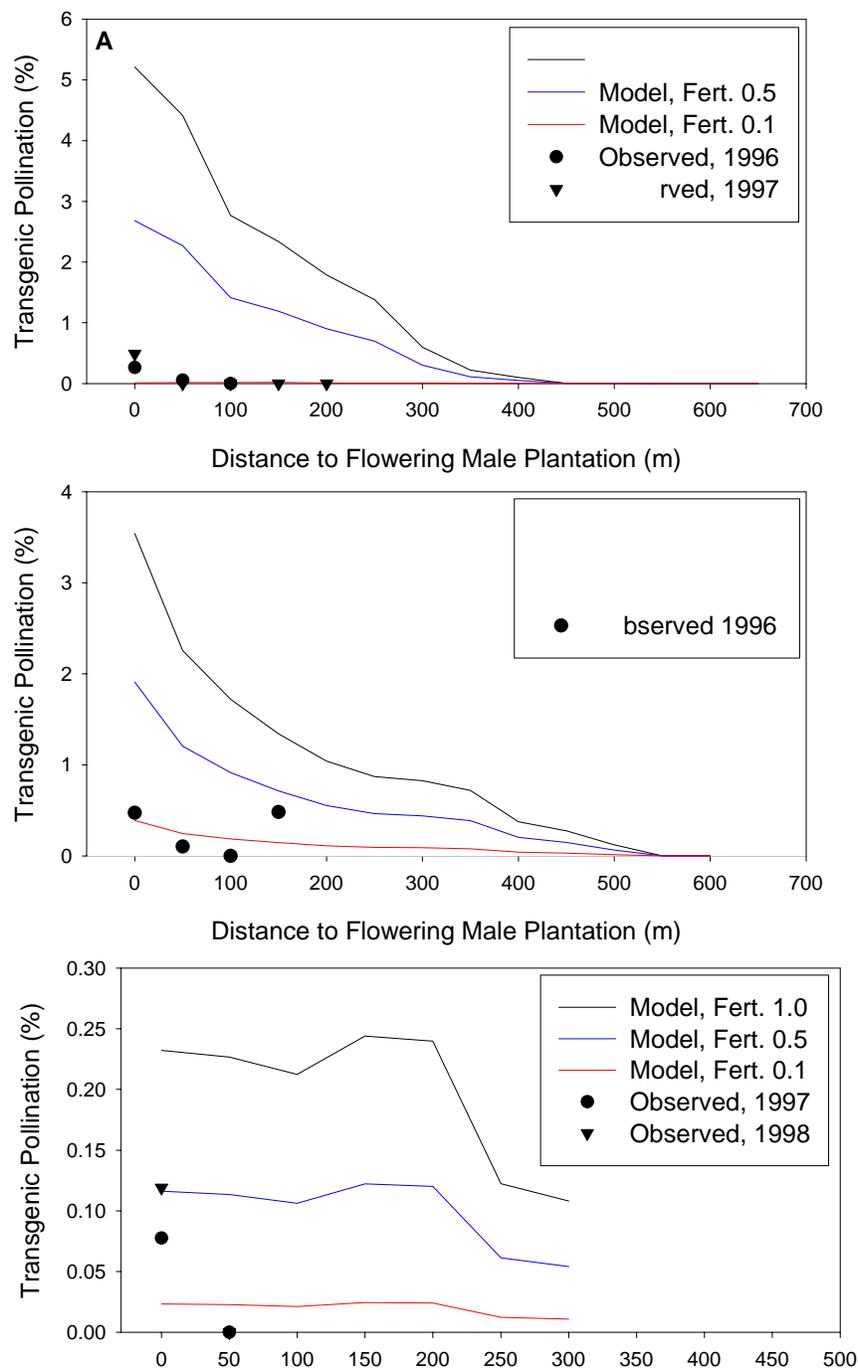
Modeled pollen flow from plantations was in the same range as observed pollen flow from existing plantations. Observed pollen flow was consistent with a plantation fertility level of 10-50% of wild tree fertility at the Columbia River site, and approximately 25% at the Willamette site (Figure 3.10A-C).

The picture for seed flow was much more variable and complicated. Most observations were below expectations at the River Ranch site, even for a modeled scenario in which plantation fertility was only 10% of wild tree fertility (Figure 3.11A). However, several observations were in excess of expectations for fully fertile plantations at the River Ranch site. Observations were consistently below expectations for all tested fertility levels at the Willamette River site, particularly at close proximity to the plantation (Figure 3.11B).

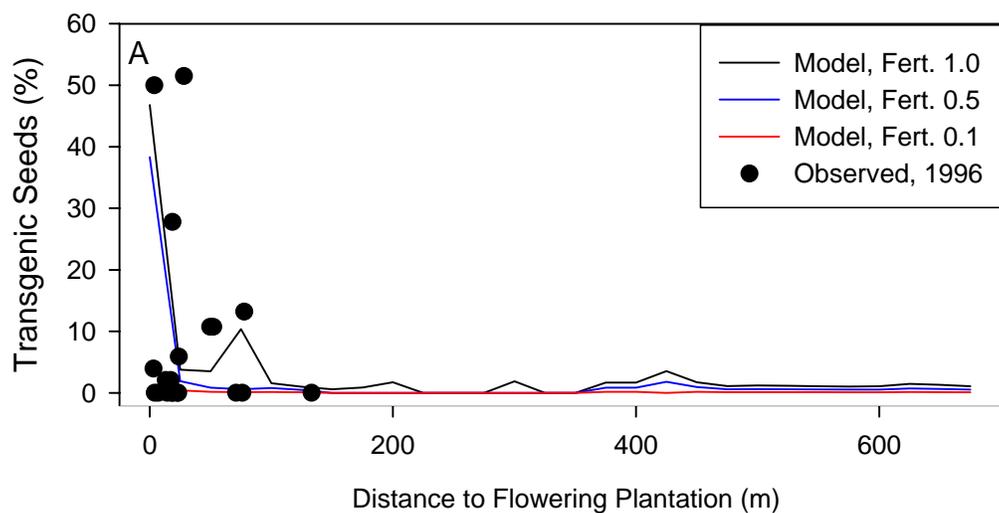
Finally, establishment of hybrids was quite low at all three sites (Table 3.9). Only one of more than 2,500 seedlings examined at Fraser River was of hybrid origin, one of more than 100,000 at the Skagit river, and no hybrid seedlings were observed among 1,200 examined on the Columbia River. For comparison, one seedling derived from Lombardy poplar was also observed at the Fraser site, and none at the other sites. These observations are well below levels expected for the landscapes examined based on STEVE model predictions. Simulations with the lower Columbia River landscape resulted in an average of 0.7% of transgenic seedlings on the landscape after one year, and an average of 1.3% transgenic seedlings over 10 years of gene flow (Chapter 4). In contrast, we observed a maximum of 0.04% plantation-derived seedlings in our surveys of wild establishment (Table 3.9), and none on the Columbia River landscape.

**Table 3.9** Establishment survey results.

Location	Area Surveyed (ha)	Plantation Area (ha)	Area of Regeneration (ha)	Number Seedlings Examined	Number hybrids (% of Examined)
Fraser River	100	118	0.05	~2500	1 (0.04)
Columbia River	2000	250	0.02	1200	0 (0)
Skagit River	56	44	0.25	~100,000	1 (0.0001)



**Figure 3.10** Comparison of modeled versus observed pollen flow from plantations. Lines represent average percentage of seeds that were transgenic for each distance class for STEVE model simulations with landscapes mimicking landscape conditions at the time of field studies. The three lines are for different levels of transgenic fertility. Points represent observed pollination as determined from paternity analyses. **A.** Clatskanie. **B.** River Ranch. **C.** Willamette.



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**Figure 3.11** Comparison of modeled versus observed seed flow from plantations. Lines represent average percentage of seeds that were transgenic for each distance class for STEVE model simulations with landscapes mimicking landscape conditions at the time of field studies. Points represent observed percentage transgenic seeds as determined from maternity analyses of seeds captured in traps near plantations. **A.** River Ranch. **B.** Willamette.

## Discussion

### **Triploid Fertility**

Triploid clones were deficient as parents in all fitness components that we examined. They produced fewer seeds, seeds were less viable, viable seeds had a shorter lifespan in

storage, and seedlings had reduced growth and survival. These results were most likely due to irregularities in meiosis for triploid parents, as illustrated by high rates of polysomy among progeny of triploids and the negative relationship between degree of seedling polysomy and growth rate. Cultivation of triploid transgenic clones is therefore likely to result in low levels of transgene flow to wild populations. However, some progeny of triploids showed no evidence of polysomy and apparently normal growth and morphology, suggesting that a low level of gene flow is possible.

Triploid poplars result from unreduced female gametes (Bradshaw and Stettler 1993), which is the most common cause of spontaneous polyploidization in animals and plants (Otto and Whitton 2000). Also, triploid poplars are apparently formed in interspecific crosses at much higher rates than in intraspecific crosses (Bradshaw and Stettler 1993), which is also a common pattern in spontaneous polyploid formation (Ramsey and Schemske 1998). Polyploid formation is a major mechanism of evolution in angiosperms, with 50% of angiosperm species showing evidence of hybrid origin (Soltis and Soltis 2000). Polyploids often exhibit enhanced variation compared to diploid progenitors, with high rates of genomic rearrangements and rapid evolution of duplicated genes (Matzke et al 1999; Otto and Whitton 2000). Also, because polyploids often display transgressive variation, such as enhanced cell size, decreased growth rate, and enhanced production of secondary compounds, they may occupy a different ecological niche than diploid progenitors (Levin 1983). This is also true of poplar triploids, which display enhanced variation in leaf morphological traits compared to diploids, and therefore may be a fertile source of adaptive variation (Wu 2000). However, the issue of fertility calls into question the evolutionary importance of triploids, because meiotic irregularities often reduce or eliminate the formation of viable gametes by triploid parents (Otto and Whitton 2000). Nevertheless, most triploids produce at least some viable gametes, and a small proportion of these can be haploid or euploid by chance alone (Ramsey and Schemske 1998). Therefore, triploids probably play an important role in evolution, and may represent an intermediate step or 'bridge' in the evolution of tetraploid species (Burton and Husband 2000; Ramsey and Schemske 1998).

## Gene Flow from Plantations

Observed gene flow from plantations was quite low, especially considering that our methods were designed to maximize the chances of detecting hybrid gene flow. We deliberately selected female trees that were as close as possible to plantations and that flowered synchronously with plantation males. For example, at the River Ranch site we analyzed seeds from eight trees (four hybrids and four *P. trichocarpa* females) that were located within the plantation borders, several hundred meters from the nearest flowering male *P. trichocarpa* trees.

The variation in reproductive success of the different male clones was probably due to a variety of factors. First, with such low levels of observed gene flow we would expect that we would fail to detect gene flow for a number of clones by chance alone. Also, the size of plantation blocks and distance from sampled trees were likely important in determining reproductive success, as we have demonstrated in more extensive analyses of gene flow in native trees (unpublished data). However, clones such as 47-174 and 46-158 had no observed pollinations of native trees, despite the large size of their plantations and proximity to the sampled mother trees at River Ranch. This may have been due to low levels of flowering in these clones, caused by moisture stress and infection by leaf rust (*Melampsora occidentalis* Jacks.) (personal observation). In contrast, clone 50-194 had relatively high reproductive success at Clatskanie, and this stand was vigorous and flowered heavily in both years of the study. Triploid clone 24-305 had high reproductive success relative to the other male clones at River Ranch, which is somewhat surprising given its low observed fertility in the controlled crosses. However, our sampling was originally designed to maximize chances of detecting gene flow for this clone, its plantation block at River Ranch was quite large, and the trees flowered vigorously, all of which favored detection of 24-305 paternity.

The pattern of higher reproductive success in hybrid-hybrid crosses than for hybrid-*P. trichocarpa* crosses should be interpreted with caution. First, we analyzed a small number of progeny from hybrid females. Also, paternity for progeny of hybrid females could not be corroborated with morphological or RAPD data, because the mother trees possess the same markers as the putative hybrid fathers. However, if this pattern is real, possible explanations include greater proximity of hybrid females than *P. trichocarpa*

females, and reduced competition from *P. trichocarpa* pollen in the interior of the plantations. It is unlikely the enhanced hybrid-hybrid reproductive success is caused by greater genetic compatibility between hybrids, because clone 47-174 had higher reproductive success in mating with a *P. trichocarpa* tree than with clone 49-177 in the controlled crosses.

It is telling that the observed levels of pollen flow from hybrids were comparable to those observed for Lombardy poplar, which was absent from the immediate sampling area at two of the sites, and present as one small wind break at Clatskanie. Lombardy is a tall male clone with a narrow crown that flowers heavily and synchronously with *P. trichocarpa* in Oregon. It probably originated as a spontaneous mutant of *Populus nigra* L. in Italy in the early 18<sup>th</sup> century, and has been in widespread cultivation throughout much of the world since that time, including in the United States since the early 19<sup>th</sup> century (Wood 1994). Our results indicate that Lombardy is quite fertile and capable of siring seeds with *P. trichocarpa* females even when the trees are separated by large distances. However, despite a long history of extensive cultivation in proximity to native trees, there is no evidence for extensive introgression of Lombardy poplar traits in native populations (personal observation; (U.S.Environmental Protection Agency 1999).

Experience with introductions of exotic poplar species in other regions may also provide an indication of the level of introgression that can be expected from hybrid poplar plantations in the Pacific northwest. *Populus alba* L., the European white poplar, was introduced to the upper midwest United States in the early 18<sup>th</sup> century, and has been propagated primarily as female clones. An extensive survey of naturalized hybrids between *P. alba* and native *P. grandidentata* and *P. tremuloides* revealed that substantial establishment of F<sub>1</sub> hybrids had occurred between 1930 and 1945, primarily in the immediate vicinity of female *P. alba* clones in areas subject to human disturbance (*e.g.*, roadsides, abandoned fields). However, despite extensive flowering overlap between hybrid and native trees, no advanced generation hybrids or backcrosses were detected, and no further hybrid establishment had occurred for the thirty years following the initial establishment phase (Spies and Barnes 1981; Spies and Barnes 1982). The lack of establishment and introgression was attributed to restricted dispersal and lack of establishment sites caused by human disturbance (Spies and Barnes 1982).

Another useful example is the introduction of *Populus deltoides* into Europe in the early 18<sup>th</sup> century, where it has hybridized with *P. nigra*, which belongs to the same section of the genus (Cagelli and Lefevre 1995). Introgression from *P. deltoides* is considered a serious threat to the conservation of *Populus nigra* genetic resources because the introduced hybrids are cultivated on a fairly large scale, they are fully interfertile with native trees, and native populations are already highly degraded due to human activities (Arens et al 1998; Heinze 1996). However, direct studies of introgression have revealed that *P. deltoides* alleles are not present at high frequencies in remnant *P. nigra* populations (Benetka et al 1999; Legionnet and Lefevre 1996) (Berthold Heinze, Personal Communication), suggesting that the threat posed by the introduced trees is less than originally anticipated.

A final example is provided by natural zones of hybridization between poplar species, which have received considerable attention over the years. *P. trichocarpa* and *P. deltoides* belong to sections of the *Populus* genus (*Tacamahaca* and *Aigeiros*, respectively) that intercross readily (Rajora and Zsuffa 1984). In fact, extensive natural hybridization occurs wherever species from these sections occur sympatrically (reviewed in (Eckenwalder 1984). However, the extent of introgression can vary substantially depending on the species involved, the environment of the hybrid zone, and the portion of the genome examined (Martinsen et al 2001). For example, Eckenwalder (1984a) examined a hybrid zone between *P. trichocarpa* and *P. fremontii* S. Watson in California and Nevada. Using representatives from putatively pure populations of each species, he constructed a hybrid index using 21 traits and 38 leaf flavonoids. The hybrids were clearly distinguished from the parental species by both morphological and chemical criteria, and there was little overlap among the three phenotypic classes. Eckenwalder (1984a) interpreted this as evidence that the hybrid swarm consisted primarily of F<sub>1</sub> individuals, and there was little evidence of introgression between these two species. However, Eckenwalder (1984a) also observed that some populations of *P. trichocarpa* in the zone of sympatry exhibited unusual morphology, and occurred in habitats more typical of *P. fremontii*. Also, there were sporadic occurrences of leaf flavones characteristic of one species in individuals of the other species. Eckenwalder (1984a,c) interpreted these anomalies as possible evidence of historic introgression between these

two species. Similar patterns of partial introgression have been documented in hybrid zones involving *P. fremontii* and *P. angustifolia* James, where most markers specific to *P. fremontii* are confined to the parental and hybrid zones, but a small proportion have introgressed long distances into *P. angustifolia* populations (Martinsen, Whitham, Turek, and Keim 2001).

Studies of a complex hybrid swarm in southern Alberta, Canada present a different picture of hybridization in *Populus*. Brayshaw (1965) first described this zone of hybridization that includes *P. trichocarpa*, *P. balsamifera* L., *P. angustifolia*, and *P. deltoides*. Based on variation in a variety of leaf characteristics, he concluded that there was continuous variation among hybrid and parental species, suggesting extensive backcrossing and advanced generation hybridization (Brayshaw 1965). Further analysis of this hybrid zone using Principle Coordinate Analysis for leaf characteristics (Rood et al 1986) and secondary compounds in bud exudates (Greenaway et al 1991) reached essentially the same conclusion.

The apparent discrepancies between the hybrid swarms in California and Canada may be due in part to methodological differences, but they may also reflect biologically significant differences in the species and environments involved in the hybrid zones. For example, Rood *et al.* (1986) used only eight foliage characteristics in their analyses, while Eckenwalder (1984a) scored 21 characters from stems, buds, and reproductive organs. In addition, Eckenwalder (1984a) used his chemical hybrid index in conjunction with morphological characters to distinguish hybrids from parents, while bud exudates were used only to confirm morphological designations in the Canadian study (Greenaway, English, Whatley, and Rood 1991).

Another explanation for the discrepancies is that the parental species in the Canadian hybrid swarm were more interfertile than those in California. However, Eckenwalder (1984b) reported 95% stainability of pollen derived from the California hybrids. In contrast, pollen viability for hybrids derived from *P. deltoides* and *P. angustifolia*, and *P. deltoides* and *P. balsamifera* were only 38% and 55% respectively (Smith 1943), so there is no evidence for reduced fertility of the California hybrids. Nevertheless, a direct test of crossability of these species would provide a more definitive answer.

Another possible explanation for greater introgression in Canada is that three of the species involved, *P. trichocarpa*, *P. angustifolia*, and *P. deltoides* are at the fringes of their natural range. Individuals of the pure parental species may therefore be poorly adapted to conditions at some sites, and hybrids with intermediate phenotypes may have a competitive advantage (Anderson 1949; Brayshaw 1965). In contrast, the California hybrid swarm was well within the ranges of both *P. trichocarpa* and *P. fremontii*. This is relevant to assessing potential spread of genes from plantations, because it supports the premise that the presence of large, locally adapted, native populations might substantially inhibit spread of plantation trees that would compete for the same ecological niche (Strauss 1999).

### **STEVE Model Validation**

Our validation analyses indicated that observed pollen flow from hybrid plantations was generally close to modeled pollen flow with a fertility level of 10% for plantation trees. This is a reasonable scenario, given the results of the controlled crosses that showed seed yield that for crosses between *P. trichocarpa* trees that was at least an order of magnitude higher than for crosses involving hybrids.

Observed seed flow was considerably lower than modeled predictions, even for 10% fertility. This may indicate that the STEVE model overestimates seed production for plantation trees. One key assumption is that seed production is uniform throughout plantations. This is clearly violated in commercial pulp plantations with close spacing, where most seed production appears to occur in border trees (personal observation). However, the main female clones present in the plantations, 49-177 and 52-225, each had reduced seed production and viability compared to wild trees in controlled crosses (Strauss, Han, James, Brunner, DiFazio, Meilan, and Sheppard 1996). Female hybrids often have poor seed production due to aborted embryos and premature splitting and abscission of catkins (Stanton and Villar 1996), which could strongly inhibit successful regeneration in the wild. A number of seeds germinated in our seed traps before collection, thus preventing us from identifying parentage. Therefore, our methods may also have discriminated against detection of underdeveloped hybrid seedlings with possibly precocious germination.

The STEVE model performed particularly poorly in predicting establishment of hybrid seedlings, overpredicting gene flow by at least an order of magnitude. Methodological problems could again account for the discrepancy. Many of the seedlings that we examined in the establishment surveys were quite young, and they were growing in highly variable microsites, which can cause substantial variation in *Populus* leaf morphology (Eckenwalder 1980). Therefore, we may have missed a substantial number of hybrids in our field surveys of establishment. We collected a random sample of 48 to 96 seedlings per survey, but this level of sampling may be inadequate for quantifying the low levels of gene flow predicted by the model. However, the STEVE model was designed to be highly conservative to avoid underestimation of potential gene flow (Chapters 2 and 5), so it is not surprising that predicted levels of gene flow are substantially higher than we observed.

## Chapter 4 Applications of the STEVE Model to Risk Assessment Scenarios

### Introduction

Transgenic risk assessment presents formidable challenges to regulators, scientists, and policy makers. There are multiple components of risk, encompassing ecological, economic, social, and ethical considerations (Mullin and Bertrand 1998; National Research Council 1983; Radosevich et al 1992). Estimation of risk requires identification of endpoints (impacts of concern to interested parties), hazards, characterization of a dose-response, and quantification of potential exposure to those hazards (National Research Council 1983). Some hazards can be readily defined and measured (*e.g.*, toxic emissions), while others can be quite subtle and unpredictable (*e.g.*, ecosystem-level nontarget impacts of an insecticidal toxin: (Raffa 2001).

Methods of risk assessment developed for toxic chemical emissions provide a useful model for transgenic risk assessment. The first step of a traditional risk assessment is usually to identify a specific hazard, then to study the hazard through dose-response experiments, assess exposure to that hazard, then formulate risk management plans for remediating or mitigating the sources of the hazard (National Research Council 2000). Risk assessment for transgenic organisms sometimes jumps straight to the exposure portion of the equation, even though specific hazards have not been adequately characterized, or perhaps even identified. This is partly because genetic engineering is often perceived to be revolutionary and preternaturally powerful, and therefore capable of substantial, unpredictable impacts (Mayer 2001; Petrie 2000; Rissler and Mellon 1996). Furthermore, some consider transgene flow to be a form of pollution because it disrupts the genetic integrity of a species (Johnson and Kirby 2001; Mayer 2001; Rissler and Mellon 1996; Thompson and Strauss 2000). According to this viewpoint, the hazard is absolute, and gene flow is essentially the entire risk equation.

Dose-response is a concept that has not been explicitly applied to questions of gene flow, but simulation models make such an analysis possible. Sensitivity analyses are analogous to dose-response experiments in that both can be used to identify thresholds for significant effects. For example, one could quantify the competitive effects of

transgenes in extensive field experiments designed to test a broad range of conditions and genotypes (*e.g.*, (Crawley et al 2001; Snow et al 2001; Stewart et al 1997). A model could be parameterized with these values and appropriate variances and a set of simulations could be performed with a range of fertility values for transgenic plantations, and a minimum fertility threshold for continuous transgene spread could be identified (Chapter 2).

In its idealized form, endpoint identification and assessment is an objective, scientific process. However, politics, ethics, sociology, and science converge in the identification of risks in the real world (Kasanmoentalib 1996; Radosevich, Ghersa, and Comstock 1992), and risk perception is colored by experiences and world view (Finucane et al 2000; Lackey 1994). Major social issues include the distribution of benefits and risks (Halweil 1999; Shrader-Frechette 1995), the role and appropriateness of intellectual property (Robinson 1999; Thompson and Strauss 2000), and inclusion of alternative world views in the decision-making process (Mayer 2001). Given the importance and complexity of endpoint characterization, elaboration of a definitive transgenic risk assessment is clearly beyond the scope of the present study. Rather, in this study we focused primarily on developing tools and methods for quantifying potential gene flow that can be applied to most future risk assessments for transgenic poplar. We have already described a spatial simulation model, STEVE, and demonstrated its utility for sensitivity assessments (Chapter 2). Here we use the model and a more realistic landscape to explore three case studies: transgenic poplars with neutral fitness, insect resistance, and herbicide resistance.

## **Methods**

### **STEVE Model Description**

The STEVE model operates on a landscape grid containing information about elevation, habitat type, and poplar populations. We derived landscape features by delineating habitat types from air photos (Allen 1999) and from a digital elevation model. We tessellated the landscape with a resolution of 0.1 ha cells (10 m x 10 m), which approximately represents the area occupied by a single large wild poplar tree. Cell sizes

for forest landscape models are often in this range for the same reason (Higgins et al 1996; Shugart 1998). Each cell contains information about habitat type (Table 2.1), elevation, and spatial location relative to cells with similar characteristics.

The simulation has an annual time step, which begins with disturbance: the creation and conversion of poplar patches at rates determined empirically from a chronosequence of air photos. Subsequently we simulate growth, reproduction, dispersal, and competition for poplar cohorts. Poplar behavior depends on location (plantation versus wild) and genotype (transgenic versus conventional). Basal area is proportional to age and number of trees, and pollen, seed, and vegetative propagule production are in turn proportional to basal area. This is all expressed on an arbitrary scale, because the main purpose of the model is to depict the relative balance between transgenic and conventional trees, so processes are driven by the proportion of each genotype rather than absolute numbers.

Pollen and seed are produced in each cell proportional to basal area of each genotype, modulated by a fecundity factor. Dispersal occurs explicitly within local ‘neighborhoods’ according to functions derived from extensive field studies (unpublished data). Also, pollen and seed production of each genotype are tallied for the whole landscape, and a proportion of seeds and seedlings are derived from a ‘background’ cloud of pollen and seeds. Vegetative propagules are likewise produced relative to basal area, but only dispersed locally.

Establishment occurs only in sites previously cleared by ‘disturbance.’ We assume that adequate propagules will be available to occupy all available establishment sites, though the number of established seedlings is varied stochastically to accommodate variation in propagule abundance and interspecific competition. Different establishment rates are allowed for seeds produced locally, seeds from the ‘background’, and vegetative propagules, and establishment of each genotype depends on relative abundance within each propagule type.

All previously established cohorts then undergo density-dependent mortality, modulated by an age-dependent carrying capacity and a competitive differential for transgenics. This competitive differential can be positive, negative, or neutral, and this is the primary means for simulating fitness effects of different transgenes. Poplar patches are not eliminated by density-dependent mortality: it is a means for simulating

intraspecific competition between genotypes. Cells must contain at least one tree following this process. Poplar patches are only eliminated by the disturbance function (above).

Finally, management activities are carried out in poplar plantations and agricultural fields. As is the prevailing management practice, poplar plantations occur as large monoclonal blocks (mean = 16 ha). Harvesting occurs when plantations surpass rotation age, and new plantations are established according to predetermined rates of establishment of transgenic, conventional, male, and female clones. Agricultural fields contain a variety of crops defined broadly based on management characteristics (annual versus perennial, tilling, grazing, herbicide treatments). Agricultural fields are also abandoned or established according to empirically determined rates, and abandoned fields become available for colonization by poplar in subsequent years.

### **Risk Assessment Simulations**

We explored probable levels of gene flow under a range of scenarios using a landscape that represents a large area of poplar cultivation on the lower Columbia River (36.8 km x 23.0 km) (Figure 2.3). We also simulated a landscape with more extensive potential poplar habitat in upland sites, as would be found in northern Washington and British Columbia. For computational efficiency in exploratory analyses, we used a test landscape (5 km x 5 km) composed of pieces of the large landscape, with similar representation of the different habitat types (Chapter 2).

Simulations were initiated with our best estimates for parameter values, but with substantial stochasticity incorporated to reflect natural variation and uncertainty (Table 4.1). We allowed stochastic variation in fertility, competitiveness, pollen flow, seed flow, vegetative establishment, and disturbance, all of which were identified as important in the sensitivity analyses (Chapter 2). Little information was available on variation in these parameters, so we generally used a standard deviation equivalent to 50% of the mean value, as described below.

**Table 4.1** Baseline conditions for risk assessment analyses.

<b>Parameter</b>	<b>Value</b>
Landscape Area	46,000 ha (2287 x 3681 pixels)
Plantation Area	2,348 ha (19.4% of landscape)
Transgenic Plantations	1,200 ha ((9.6% of landscape)
Plantation Rotation	12 years, even aged
Plantation Density	1,100 trees/ha
Plantation Sex Ratio	50% female, by area
Transgenic Fertility	0.5
Transgenic Competitive Advantage	0
Initiation of flowering, plantations	5 years
Initiation of flowering, wild	10 years
Pollen Dispersal	Neighborhood, 440m,50%; slope, -0.007; intercept, 0.67;
Seed Dispersal	Neighborhood, 220m,90%; slope, -0.05; intercept, 0.9;
Vegetative Dispersal	Neighborhood, 220m,100%; slope, -0.1; intercept, 0.6;
Phenology Classes	3 (72% compatible)
Wind	No influence of wind on dispersal
Maximum Establishment Density	20 seedlings/m <sup>2</sup>
Density-Dependent Mortality	Slope, -0.33
Basal Area Increase	Slope, 0.04
Establishment Rates	Empirical (Figure 2.7)
Maximum Establishment, Agricultural Edges	0.05 ha

We simulated cultivation of transgenics with neutral fitness effects in the wild and various levels of fertility. In addition, we explicitly simulated fitness effects of two of the most prominent transgenic traits in forestry: insect resistance and herbicide resistance.

We performed 30 repetitions of most risk assessment scenarios and calculated the mean and 99% confidence interval of the response (transgene flow) through time.

#### *Stochasticity in Fertility*

Fertility was varied in two ways: base fertility was varied among new poplar cells and plantation blocks to reflect differences among genotypes. In addition, fertility was varied annually to reflect environmental influences (*e.g.*, weather, interspecific competition). Annual variation can be cumulative (*i.e.*, changes persist from year to year), or independent (fecundity is reset to base value annually). Annual variation is not allowed for completely sterile trees, but fecundity can be restored with a probability and rate determined by the user, at which point annual variation may ensue.

#### *Stochasticity in Competitiveness*

Variation in the transgenic competitive differential occurs simultaneously in space and time to reflect influences of factors such as weather, microsite, and interspecific interactions. The competitive deviate is sampled from a normal distribution according to a user-defined standard deviation.

#### *Stochasticity in Disturbance*

We varied rates of disturbance annually by sampling transition probabilities from a negative exponential distribution, with a mean determined by the empirically determined transition rates (Chapter 2). This mimics the natural process of poplar establishment, which consists primarily of rare bursts of establishment in response to large-scale disturbances (primarily due to flooding) (*e.g.*, (Braatne et al 1996)).

#### *Pollination and Establishment*

We expect that gene flow by pollen, seeds, and vegetative propagules will vary spatially due to effects of local populations, weather, topography, and other factors not

explicitly considered in the model. We therefore allowed random variation in rates of background pollination for each female. Similarly, we allowed variation in proportion of establishment from local seed, background seed, and vegetative propagules for each establishment site. All parameters were sampled from normal distributions with user-defined standard deviations.

## **Insect Resistance Scenario**

Poplar trees are susceptible to attack by a wide variety of herbivores, including leaf-eating chrysomelid beetles (Dickmann and Stuart 1983). One of the scenarios that we examined was the cultivation of insect-resistant transgenics (*e.g.*, trees containing the Bt endotoxin gene, which has been used to create beetle-resistant poplar trees: Meilan *et al.* 2000). We sought to examine how mean growth enhancements determined in field trials would translate to actual fitness advantages and transgenic gene flow, given variation in insect pressure and growth enhancement in the wild.

### *Source Data*

We obtained data on insect resistance of transgenic hybrid poplars containing a gene encoding a modified version of the Cry3a endotoxin from *Bacillus thuringiensis* (Bt). The growth advantage was approximately 13% for transgenic versus conventional clones of the same genotype (Meilan *et al.* 2000). These studies were carried out under nearly ideal moisture and nutrient conditions, but with high insect pressure. These trials therefore provide idealized estimates of the short-term advantage that this transgene might confer on trees growing in the wild (Strauss *et al.* 2001).

### *Implementation in the Model*

We simulated insect attack in the wild as a stochastic process that affected patches of trees up to 0.5 ha in size. Creation of an insect attack patch occurred with a predetermined probability, and was independent between years (*i.e.*, insects disappeared and reappeared annually at random locations). This resulted in a range of insect pressures on the landscape (Figure 4.1). The attack was manifested as the relative growth advantage of a transgenic tree at that site of attack, and this value was determined by



### *Assumptions and Limitations*

1. *Insect attack occurs in discrete patches, and there is little correlation in location or intensity of attack between years when insect pressure is low on the landscape.* Insect outbreaks in the model varied in size from 0.01 to 0.5 ha, though patches coalesced into larger sizes at higher insect population levels (Figure 4.1). Furthermore, attack in one year did not increase the probability of attack in a subsequent year for a particular location. This is surely a simplification, because the insects pupate, and emerging larvae inflict a large proportion of the ensuing damage (Floate et al 1993). Effects are therefore likely to appear in the same location for multiple years. However, other factors such as adult dispersal rates, variation in overwinter mortality, and development of host defenses, are largely unknown, so this is a reasonable null hypothesis.
2. *Variation in competitive advantage due to insect resistance is uniformly and randomly spread among adult trees.* We allowed for random variation in fitness advantage at the level of individual cells, so that fitness advantage could vary from nearly 0 to the maximum value over a small area. In fact, there would likely be some spatial correlation in intensity of attack, and therefore in the average fitness advantage. However, local variation in insect pressure can be considerable, and innate host resistance is under strong genetic polygenic control, and therefore is likely to segregate in wild populations largely independently of spatial location, given the high gene flow rates for this species (Chapter 3; (James and Newcombe 2000b).

### **Glyphosate Tolerance Scenario**

One of the main challenges facing hybrid poplar growers is weed control, and herbicides are a primary means for achieving this. Therefore, herbicide tolerance is one of the most important traits being pursued by genetic engineers. We have explicitly simulated gene flow from plantations containing transgenic trees that are tolerant for the herbicide glyphosate (active ingredient of Roundup®). Glyphosate tolerance should only impart a fitness advantage in environments where the target herbicide is used as an important means of controlling the transgenic tree. Herbicides are typically not sprayed

outside of managed landscapes, so we focused on establishment in and near agricultural fields and poplar plantations for this scenario. These are also the locations where the greatest economic impacts of transgenic poplars might be manifested, so gene flow to managed lands is likely to be a key concern of regulators and the public.

#### *Source Data*

We obtained extensive data from field tests of herbicide-resistant transgenic poplars (*e.g.*, (Meilan, Ma, Eaton, Miller, Crockett, DiFazio, and Strauss 2000)). These trials provide data on the advantage conferred by the transgene in the presence of glyphosate, fitness costs of the trait in the absence of the herbicide, and of the variation expected in the transgenic phenotype. Data from the field trials provided a starting point for values to be used in the simulations, though they are not directly representative of transgene effects in the wild.

We obtained data on crops and herbicide usage in Clatsop and Columbia counties, both of which are included in our focus area on the lower Columbia River in Oregon (National Agricultural Statistics Service 1999). We assigned management regimes to fields based on these statistics (Table 4.2).

We are not aware of data on natural establishment of poplar in agricultural or plantation settings. However, we conducted a survey of resource professionals to gather data on management and control of poplar trees (Appendix). Forty percent of respondents indicated that cottonwoods commonly establish in agricultural fields, 48% commonly observed them on field margins, and 19% observed them in conifer plantations (Appendix). We conclude that establishment at field margins should be more common than in other settings, and we simulated a wide range of establishment rates and tracked the area of agricultural fields and margins occupied by poplar over time in each scenario. In addition, we analyzed establishment of poplar in abandoned agricultural fields using the air photo chronosequence, and we assume that this establishment rate represents the maximum expected in a field or plantation.

### *Implementation in the Model*

We allowed poplar establishment on edges of all agricultural fields and in interiors of fields that were not subjected to regular tilling or grazing (*e.g.*, nurseries, Christmas tree farms, plantations). The maximum size of establishment at field edges was 0.05 ha, and 0.01 ha in field interiors. Each year, poplars in agricultural settings were susceptible to stochastic mortality at user-defined rates, as well as mortality due to herbicide spraying. Stochastic mortality represents disappearance of the trees due to anthropogenic mechanisms such as manual removal or spraying with an herbicide other than glyphosate, as well as natural mortality due to interspecific competition and/or disturbance. In addition, density-dependent mortality within cells occurred in the same manner as for wild habitats, with a selective differential for trees sprayed with glyphosate. Herbicide spraying was simulated annually for all fields that were designated to receive treatment, based on herbicide use patterns documented by the USDA (National Agricultural Statistics Service 1999). We simulated herbicide effects up to a maximum poplar age because it is unlikely that control of large trees would be attempted solely with glyphosate, and herbicide treatment is more likely shortly after establishment.

Default conditions for the sensitivity analyses and risk assessment simulations are summarized in Table 4.2.

We expected very little effect of herbicide tolerance in unmanaged lands because we allowed no selective advantage in environments where the herbicide was not used. Therefore, in addition to examining effects on total transgene flow as for other scenarios, we also focused on establishment in managed lands for the herbicide tolerance scenario. In this case the response was the proportion of all managed lands that contained transgenic poplars. More specifically, the response was the percentage of all 100 m<sup>2</sup> cells with the ‘agriculture’ habitat type that contained poplar volunteers of any age. We focused on poplar trees of all ages rather than just mature trees on the assumption that poplars of any age in agricultural fields could be economically detrimental. This is referred to as ‘agricultural establishment’.

**Table 4.2** Baseline conditions for glyphosate tolerance simulations. Starting conditions for other parameters were equivalent to those of the risk assessment simulations (Table 4.1).

<b>Parameter</b>	<b>Value</b>
Transgenic Herbicide Competitiveness	0.5 (0.25 sd)
Transgenic Herbicide Resistance	0.9 (0.1 sd)
Conventional Herbicide Resistance	0.05 (0.025 sd)
Ag Interior Establishment	0.0001
Ag Interior Mortality	0.2
Ag Edge Establishment	0.0001
Ag Edge Mortality	0.1
Max Age of Spraying	3
Row Crop Spray Probability	0.5
Pature Spray Probability	0.01
Grass/Hay Spray Probability	0.01
Plantation Spray Probability	0.5

*Assumptions and Limitations*

1. *Land use in managed lands is fixed through time.* The proportions of area in each land use type are fixed at model initiation, though fields are occasionally abandoned and allowed to revert to wild conditions, and new agricultural fields and plantations are occasionally established at rates based on observations from air photos.
2. *Settings exist where glyphosate is the primary means of controlling volunteer poplar trees.* In fact, our survey results indicate that glyphosate is often a prominent component of weed control regimes in a variety of settings, but it is rarely the sole method (Appendix). It is usually used in conjunction with other herbicides and control methods, and poplar is usually susceptible to these other forms of control. We have accommodated these other control measures by allowing relatively high mortality rates due to factors other than glyphosate (Table 4.2). Glyphosate can be virtually the sole measure of weed control in some settings, such as on public lands in Canada or some roadsides where use of other more toxic herbicides is strictly controlled.
3. *There is a limited period during which glyphosate is an effective control measure for poplar trees.* We assume that after a certain number of years, poplar will attain a stature that reduces the efficiency of control with standard herbicide spraying. At this point, mechanical control measures are more likely (J.S. Ketchum, personal communication, 2001). Also, herbicide spraying is much more likely in the first few years following establishment in a plantation, because weed control should diminish as the plantation canopy closes and weed establishment declines.
4. *Large poplar trees can persist in some managed settings.* Clearly poplar trees will be removed by some means in fields that are tilled and planted annually, and this typically involves some means other than spraying of glyphosate. Also, grazing is quite effective at removing poplar volunteers (Crouch 1979). Therefore, the only settings in which poplar establishment is allowed are those that are not tilled or grazed regularly: edges of all fields, and interiors of fields with lightly managed perennial crops, or tree plantations.

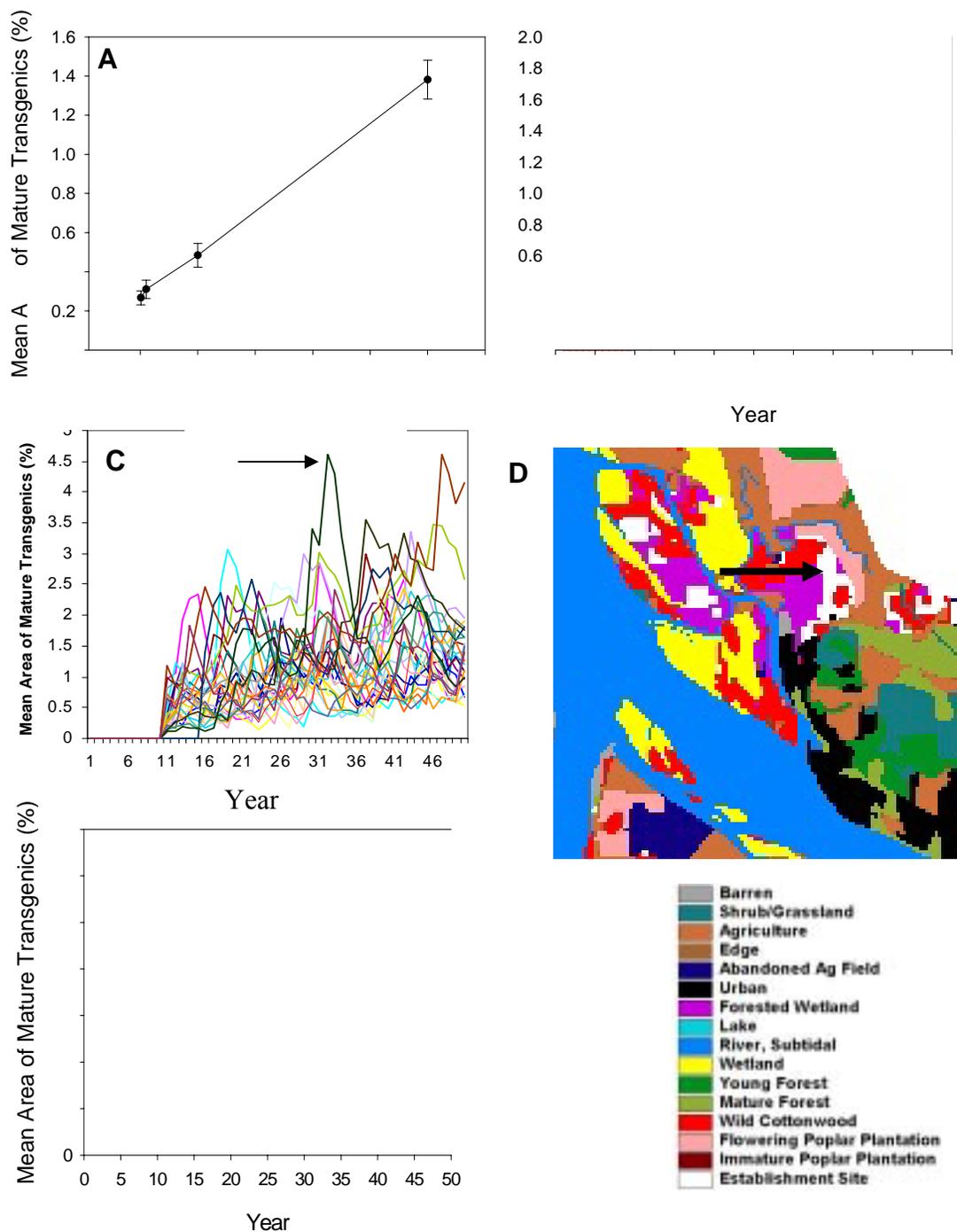
5. *Rates of establishment in field edges and interiors of lightly managed fields can be approximated by establishment rates in abandoned fields.* We use abandoned field establishment rates in the absence of direct data on establishment in these other settings. The resolution of our air photo survey was inadequate for measuring establishment of individual trees at field edges. Also, it was unknown which specific fields could be considered ‘lightly managed’ at the time of the air photos. Therefore, abandoned field establishment rates represent a conservative starting estimate of rates to be expected in and around managed lands, because these rates are likely overestimated.

## **Results**

### **Neutral Trait**

Transgene flow averaged 1.4% over the final 25 years of the risk assessment simulations with commercial scale cultivation and neutral transgenic competitiveness in the wild (Figure 4.2A). The maximum upper 99% confidence interval for transgene flow was 1.95%, and there was substantial variation among years (Figure 4.2B). After 50 years, transgenics constituted 0.38% of the basal area of wild poplar trees (99% CI 0.21 to 0.55%). There was a distinctive peak in gene flow at year 31, which corresponded to a peak in transgenic establishment 10 years earlier (Figure 4.2C). This peak was primarily due to a large establishment event adjacent to a mature female plantation in one of the thirty repetitions (Figure 4.2D), but this peak of gene flow was transient, disappearing shortly after the trees reached maturity (Figure 4.2C).

Transgenics with reduced fertility had much lower levels of gene flow, with little difference for fertilities ranging from 0.1% to 10% of conventional fertility (Figure 4.2A). For example, transgene flow averaged 0.27% for scenarios with 0.1% fertility, with a maximum upper 99% confidence interval of 0.44% (Figure 4.2B). However, there was an apparent gradual but continuous increase in gene flow through time with reduced fertility (Figure 4.2B). After 50 years, transgenics accounted for 0.16% of wild poplar basal area (99% CI 0.1 to 0.22%).



**Figure 4.2** Results of risk assessment simulations with neutral transgenes. **A.** Transgene flow with varying levels of fertility. **B.** Gene flow by year. **C.** Gene flow by year for individual repetitions. Arrow indicates pulse in transgene flow in year 31. **D.** Map of establishment adjacent to mature female transgenic plantation (indicated by arrow) that accounts for pulse in establishment for rep in C. **E.** Transgene flow in Northern landscape, characterized by enhanced establishment of poplar at higher elevations. Data are shown for default disturbance rates and reduced disturbance.

Maximum gene flow is potentially much greater for a landscape with a larger area available for establishment (*e.g.*, a landscape in northern Washington state where poplar can become established in uplands). For example, if we allowed poplar establishment above 15 m elevation (“Northern Landscape” scenario), gene flow could reach as high as 12.4% within 25 years, though this value rapidly declined to around 2% gene flow, as density-dependent mortality occurred, and poplar populations approached an equilibrium (Figure 4.2E). Maximum values were not as extreme with lower disturbance rates, and gene flow still converged to around 2% by year 50 (Figure 4.2E).

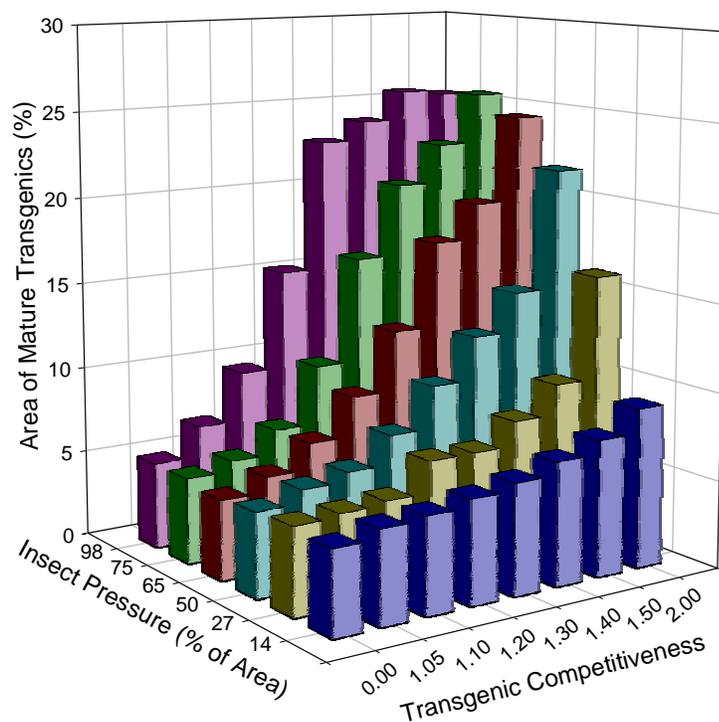
## **Insect Resistance Scenario**

### *Test Landscape*

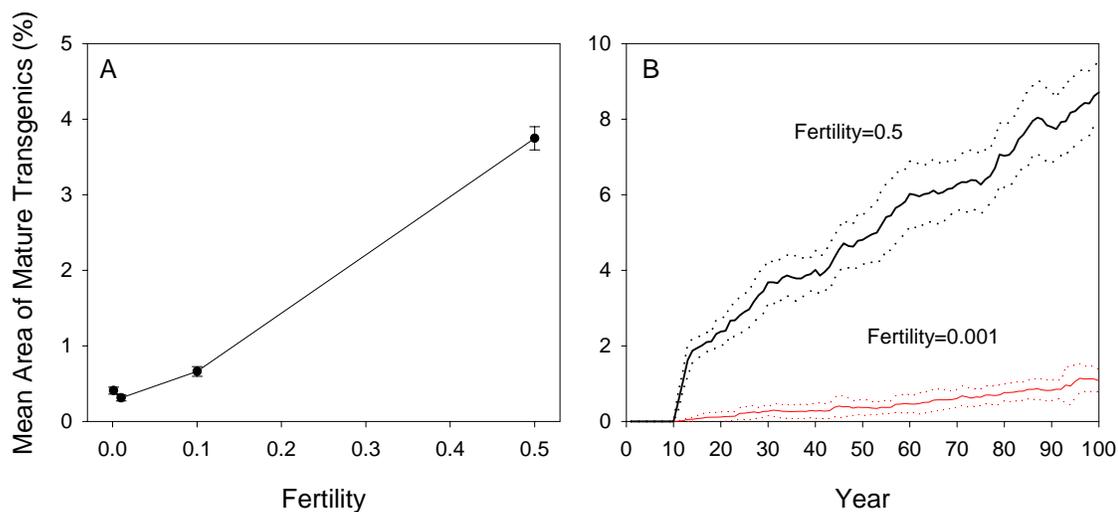
We tested a wide range of theoretical average competitive advantages conferred by the transgenic insect resistance gene. There was a strong interaction with the amount of insect pressure present on the landscape (Figure 4.3). Even at an extremely high transgenic advantage (mean of 100% growth enhancement due to insect protection,  $\alpha=2.0$ ), there was only a marginal increase in transgene flow when 14% of the wild poplar stands were subjected to insect herbivory. As insect pressure increased, the level of transgene flow was progressively enhanced: with a mean advantage as low as 10%, some enhancement of transgene flow was observed when 98% of trees were subject to insect attack (Figure 4.3).

### *Full Landscape*

Insect-resistant transgenics had gene flow averaging 3.7% from year 25 to 50 for simulations on the full landscape, with 50% of the trees on the landscape subject to herbivory and an average competitive advantage of 30% (Figure 4.4A). The maximum upper 99% confidence interval observed over a 100 year simulation was 9.5%, but gene flow increased steadily through time (Figure 4.4B). After 50 years, transgenic insect-resistant trees constituted 0.74% of the basal area of wild poplar (99% CI 0.52 to 0.96%). As in the neutral simulations, reduced transgenic fertility resulted in much lower gene flow, with little difference across a broad range of fertilities, averaging 0.4% for



**Figure 4.3** Transgene flow under a variety of insect pressures and mean levels of transgenic advantage. Substantial insect pressure in the wild, and high fertility, is required before transgenic advantage can have a substantial impact on transgene flow.



**Figure 4.4** Insect resistance simulations for the full lower Columbia River landscape. **A.** Transgene flow at different levels of fertility. **B.** Transgene flow through time at two levels of fertility.

transgenics with 0.1% fertility (Figure 4.4A), representing 0.25% of the basal area of wild poplar (99% CI 0.15 to 0.35%). However, gene flow increased continuously through time for reduced fertility transgenics as well (Figure 4.4B).

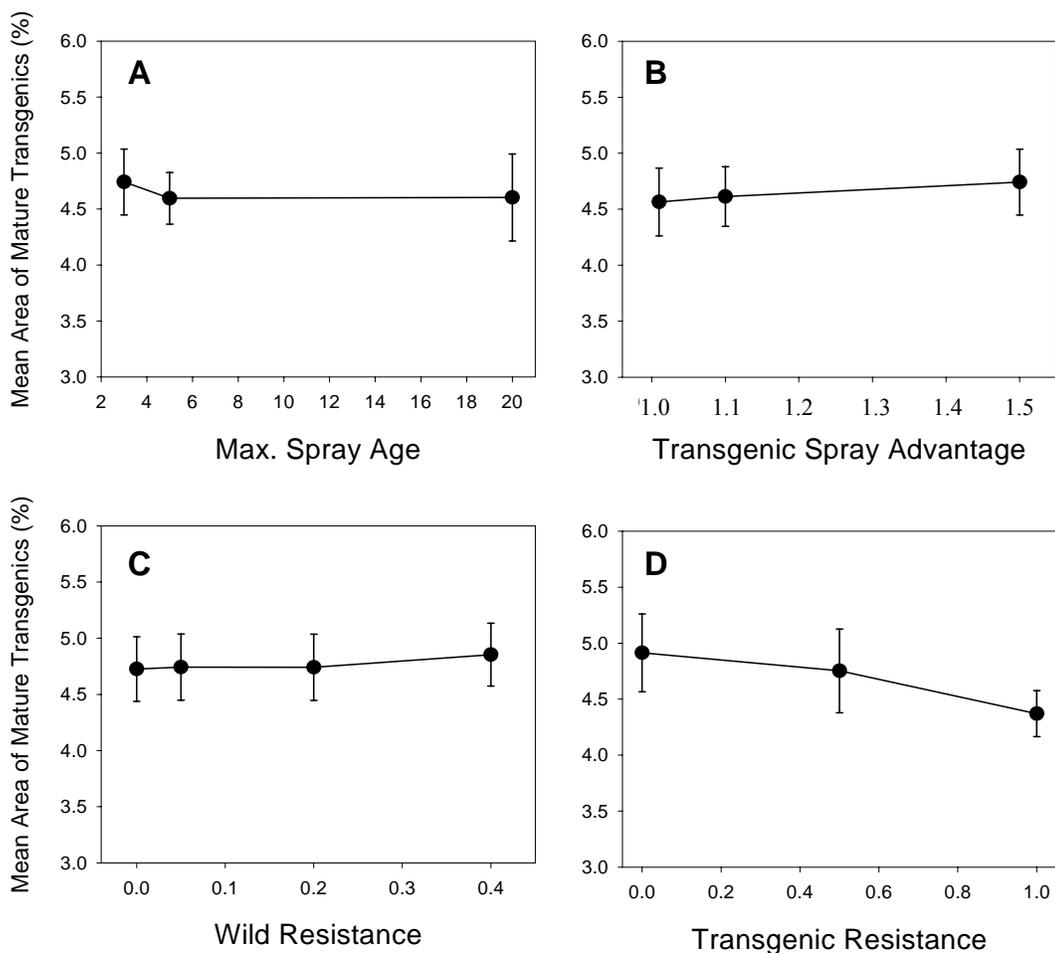
## **Glyphosate Tolerance Scenario**

### *Test Landscape*

Glyphosate tolerance had very little effect on overall levels of transgene flow, even across a very broad range of parameter values (Figure 4.5A-D), and transgene flow was similar to that observed for neutral transgene on the test landscape (i.e., 4.5%, Chapter 2). This was as expected, because a competitive advantage was only allowed in places where glyphosate was used (i.e., managed lands: agricultural fields and plantations), which constituted a small minority of available poplar habitat annually. We therefore looked in more detail at establishment in managed lands for this scenario. Overall establishment in managed lands was quite low for all scenarios tested, with maximum establishment totaling less than 0.5% of all managed lands for most scenarios tested (Figures 4.6 and 4.7). The parameter with the largest effect on managed land establishment was the establishment rate in the interiors of fields (Figure 4.6A). In contrast, establishment rate at the edges of fields (Figure 4.6C), and mortality rates (Figures 4.6B,D) had minor effects on agricultural establishment. Among traits specifically related to glyphosate resistance, the age of effective spraying had a small effect on agricultural establishment (Figure 4.7A), and transgenic resistance level had a somewhat stronger effect on agricultural establishment (Figure 4.7D). Finally, competitive advantage of transgenics after spraying and wild resistance level each had no effect (Figures 4.7B and C).

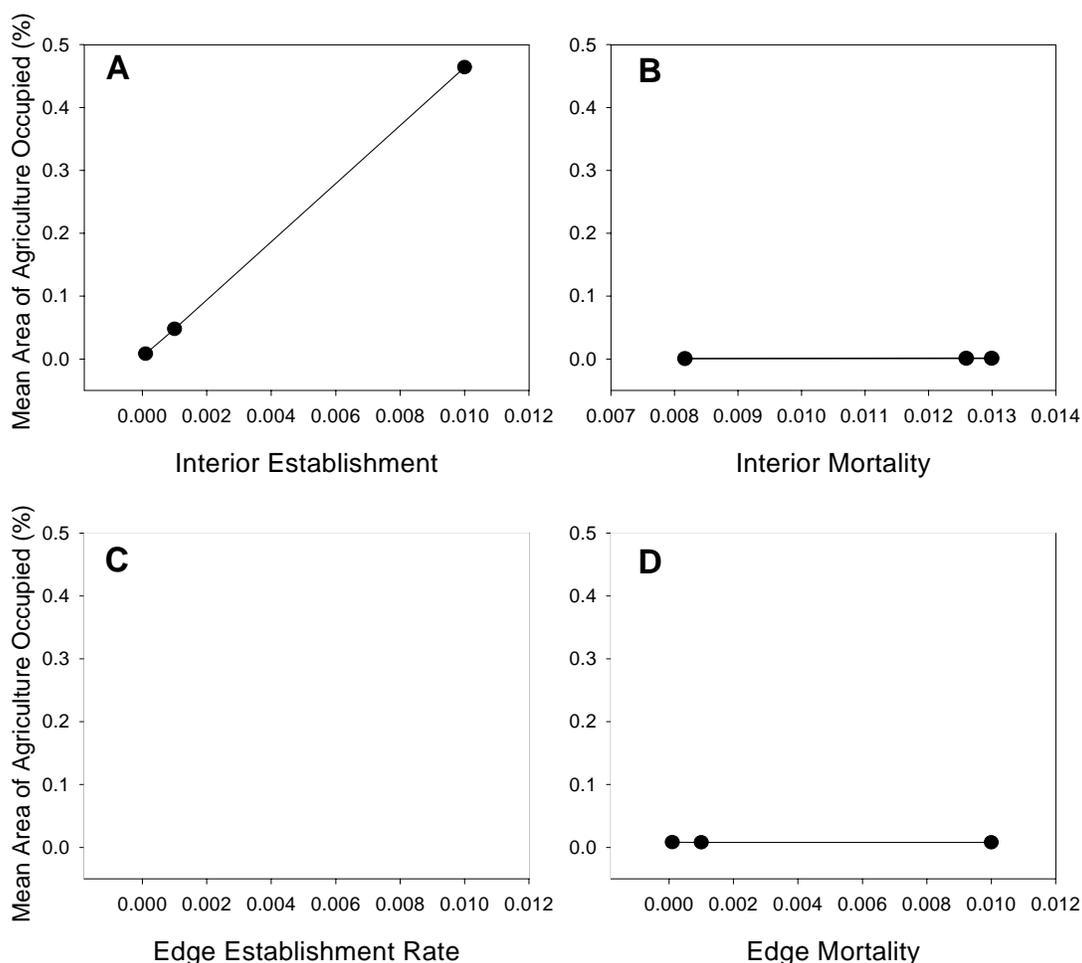
### *Full Landscape*

As we observed for the test landscape, transgene flow for herbicide tolerance transgenes was not different from overall neutral transgene flow. This was true for the lower Columbia River landscape as well as a landscape in which establishment was allowed in uplands (Northern landscape) (Figure 4.8A). Cultivation of herbicide



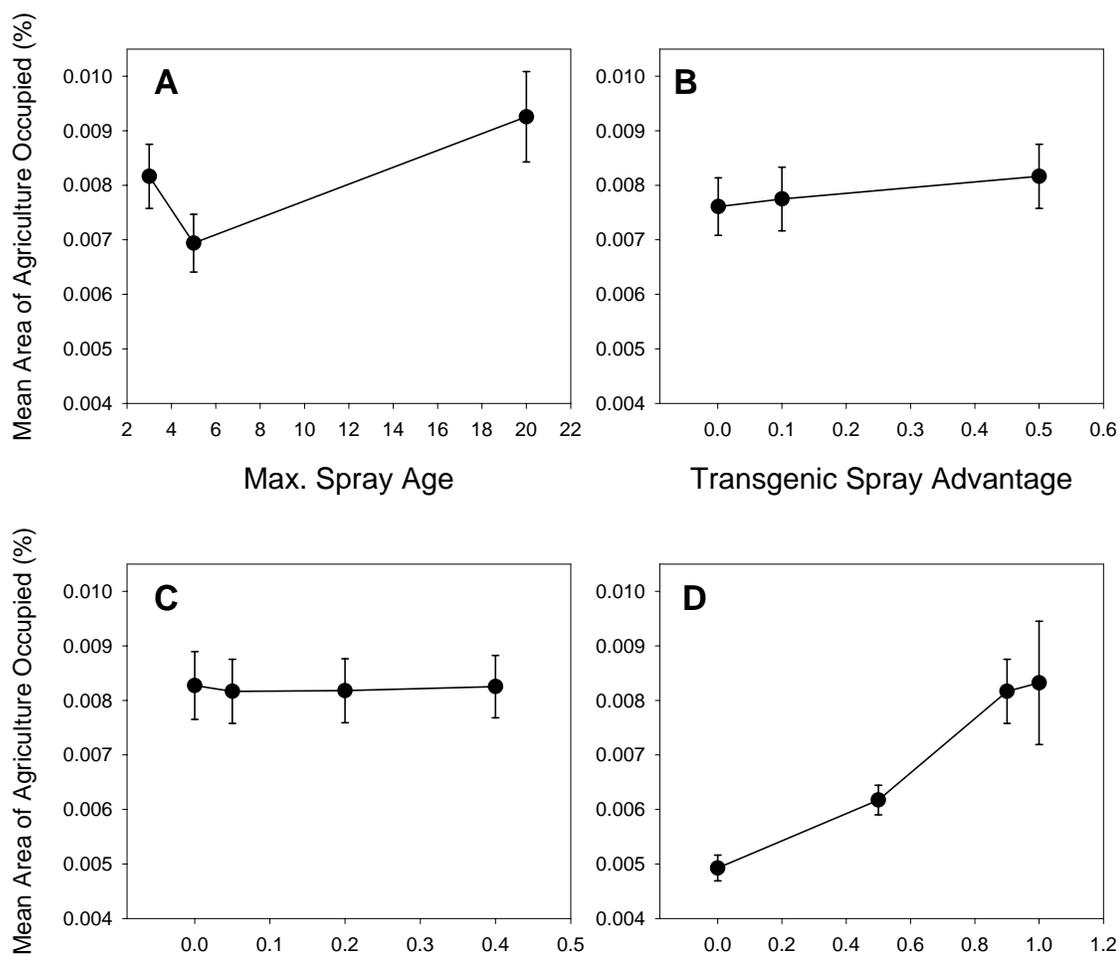
**Figure 4.5** Response of transgene flow on test landscape to changes in parameters in glyphosate resistance scenario. **A.** Maximum age at which poplars are controlled by spraying. **B.** Relative competitive advantage of transgenics when sprayed. **C.** Resistance of wild trees to spraying (probability of survival). **D.** Resistance of transgenic trees to spraying.

tolerance transgenics on a commercial scale resulted in 1.38% gene flow on average, with a maximum of 2% over a 50 year period. Transgenics with 1% fertility had 0.35% gene flow, with a maximum of 0.46%. Gene flow was considerably higher for the Northern landscape, averaging 4.9%, with a maximum of 7.1% within 50 years. Reduced fertility transgenics averaged only 0.5% gene flow on the Northern landscape, with a maximum of 0.7%.



**Figure 4.6** Response of agricultural establishment (percentage of area of managed land containing transgenic poplars) on test landscape to changes in establishment and mortality parameters. Data are from the same model runs as figure 4.5. **A.** Rate of establishment in interior of fields. **B.** Rate of stochastic mortality of poplar established within fields. **C.** Rate of establishment at edges of fields. **D.** Rate of stochastic mortality of poplar established at edges of fields.

Agricultural establishment was quite low for the lower Columbia River landscape, averaging less than 0.1% of managed fields. Agricultural establishment averaged nearly 1% for the Northern landscape, which had a larger proportion of managed lands not subjected to regular tilling and grazing. Transgenics with 1% fertility had gene flow reduced by more than half on each landscape (Figure 4.8B).



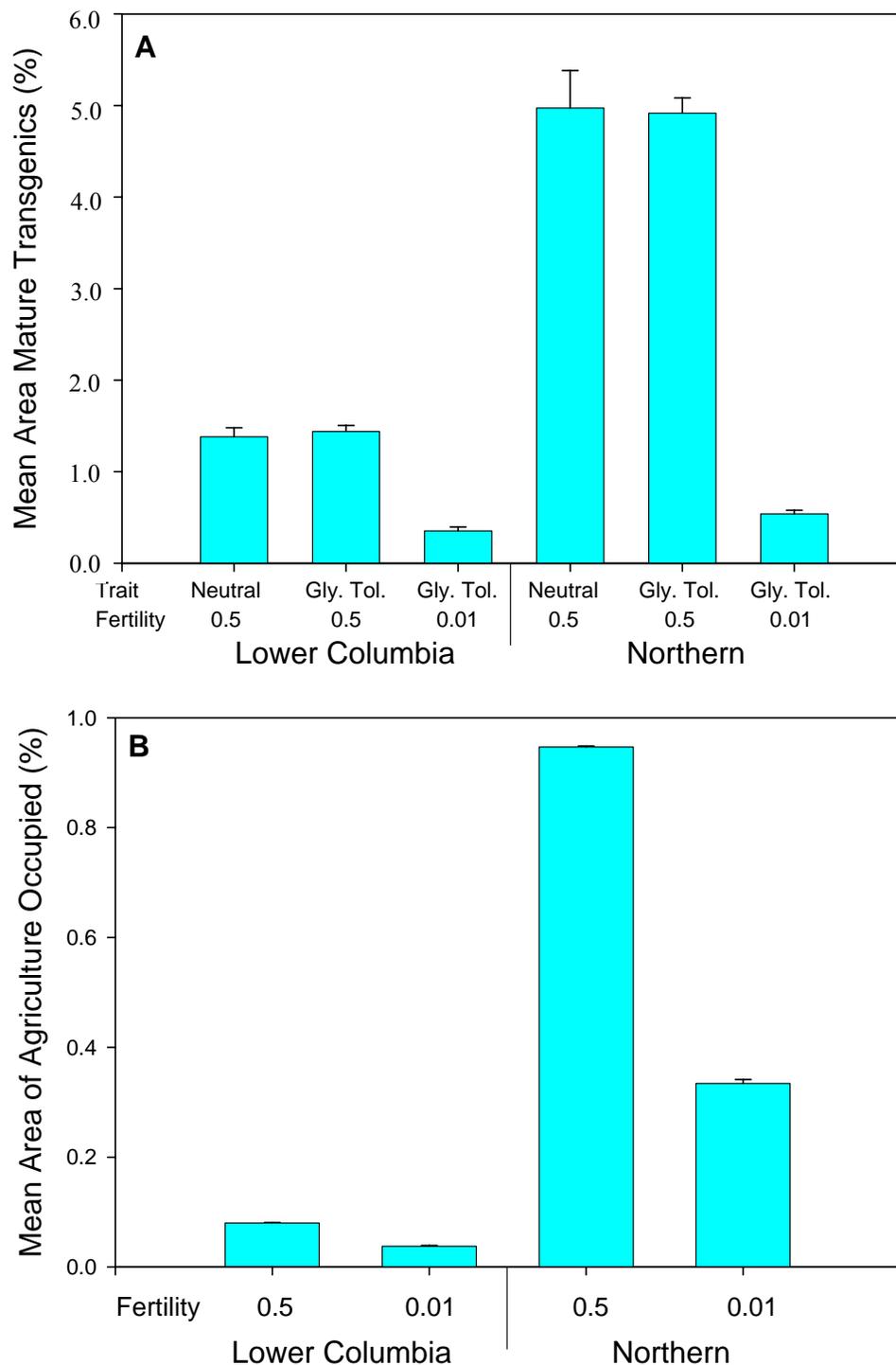
**Figure 4.7** Response of agricultural establishment (percentage of area of managed land containing transgenic poplars) on test landscape to changes in parameters in glyphosate resistance scenario. Data are from the same model runs as figure 4.5. **A.** Maximum age at which poplars are controlled by spraying. **B.** Relative competitive advantage of transgenics when sprayed. **C.** Resistance of wild trees to spraying (probability of survival). **D.** Resistance of transgenic trees to spraying.

## **Discussion**

### **Neutral Transgene**

There is no *a priori* reason to believe that a selectively neutral transgene would persist and spread in large wild populations in the absence of substantial, persistent gene flow from plantations (Ellstrand and Hoffman 1990; Gabriel 1993; Tomiuk and Loeschke 1993; Tømmerås et al 1996). Horticultural varieties of poplar have long been grown in close proximity to interfertile wild relatives, yet minimal gene flow has resulted, even in cases where native populations have been decimated by human activities. For example, introduced varieties of *Populus deltoides* and *P. deltoides* x *P. nigra* have been cultivated in Europe for over 200 years, yet there has been relatively little introgression of *P. deltoides* alleles into the degraded *P. nigra* populations (Heinze 1998). Our own studies revealed levels of pollen flow from small stands of Lombardy poplar (*Populus nigra* cv. *italica* Duroi) comparable to gene flow from hybrid poplar plantations (Chapter 3). Lombardy poplar has been cultivated in North America for over 200 years, and was quite widespread in the late 19<sup>th</sup> century (Wood 1994). Nonetheless, there is little evidence of extensive introgression in native populations, despite the striking appearance of hybrids due to the columnar crown form and distinctive leaf morphology of the Lombardy cultivar (U.S.Environmental Protection Agency 1999); personal observation). Finally, natural hybrid zones have existed between sympatric native poplar species for millennia, yet these species have retained their identities, with remarkably little introgression (Eckenwalder 1984; Martinsen et al 2001). One possible explanation is the species have distinct ecophysiological characteristics, and therefore occupy distinct niches, and the intermediate hybrids have inferior fitness in either of the parental habitats (Anderson 1949; Eckenwalder 1984); but see (Arnold 1997).

Our findings with the STEVE model generally support the expectations of low levels of gene flow with a neutral transgene. Transgene flow stabilized between 1 and 2% after 25 years for neutral transgenics with fertility levels about 50% those of wild trees, a value approximating the ratio of conventional to transgenic seed production on this landscape.



**Figure 4.8** Risk assessment simulations for herbicide tolerance transgenics for the full lower Columbia River landscape and the northern landscape with broader poplar establishment opportunities. **A.** Transgene flow for cultivation of neutral transgenics (Neut.), glyphosate-resistance transgenics (gly), and glyphosate resistance transgenics with reduced fertility. **B.** Agricultural establishment for glyphosate tolerance transgenics, by fertility level.

Also, this gene flow estimate may not provide an accurate estimate of exposure, because our definition of gene flow has a very coarse resolution (100 m<sup>2</sup> area) and gives equal weight to all reproductively mature trees regardless of age or size. A more accurate estimate of magnitude of effective gene flow is the basal area of transgenic trees relative to basal area of native trees. By this measure, transgenics constituted less than 0.5% of wild populations after 50 years of simulation. Gene flow values were considerably less when transgenic fertility was reduced to 10% of wild fertility or less.

In some respects, this is probably an overestimate of potential gene flow for the Columbia River landscape. The baseline conditions for risk assessment simulations allowed for plantation fertility that was 50% of wild fertility, and plantation trees were equally competitive with wild trees. Our field observations indicated that effective male fertility matched modeled plantation fertility closer to 10%, and observed seed flow was well below expectations for 10% fertility (Chapter 3). Also, the reduced levels of gene flow observed with low fertility transgenics were almost certainly overestimates due to the STEVE model structure (as described in the fertility section, Chapter 2).

The model effectively allowed transgenic establishment in all new cohorts, regardless of location on the landscape. This background seed establishment accounted for the vast majority of gene flow in most simulations. In reality, our field establishment surveys detected no establishment of plantation-derived seedlings at multiple locations, including a site of high disturbance with a long history of hybrid poplar cultivation (Fraser River, Chapter 3). Finally, modeled disturbance rates probably exceeded actual disturbance rates for this landscape, thus allowing more opportunities for establishment of transgenics. For the purpose of risk assessment, these gene flow estimates can therefore be considered highly conservative (*i.e.*, overestimates of risk).

Gene flow estimates had remarkably small 99% confidence intervals, especially considering the levels of stochasticity incorporated into the simulations. We allowed substantial stochastic variation in fertility, competitiveness, background pollination, and establishment parameters, all of which showed significant effects on transgene flow in sensitivity analyses. However, the relatively small amount of variation suggests that some key model parameters were deterministic in the risk assessment simulations. Future simulations should explore the implications of allowing stochastic variation in

individual pollination and establishment events. In the current version of STEVE, transgenic pollination and establishment are deterministic in that the production of transgenic seeds or seedlings is a direct function of the proportion of transgenic pollen arriving at stigmata, and the proportion of transgenic propagules arriving at establishment sites, respectively. An alternative is to use these proportions as probabilities of success of individual transgenic propagules. This would also improve the performance of the model in simulating low levels of fertility.

Another important issue is the scope of inference for these simulations. Most simulations took place on a landscape that was based closely on the lower Columbia River at the border of Oregon and Washington, an area of intensive hybrid poplar cultivation. Simulations with relaxed assumptions about habitat requirements resulted in a much different pattern of gene flow, with an initial, strong peak as poplars colonized upland areas, followed by a gradual decline as density-dependent mortality led to dominance by native poplars. Final gene flow rates approached those of the Columbia River scenario, and again reflected the approximate proportion of transgenic seeds produced on the landscape. Because of the overwhelming influence of background establishment, the equilibrium level of neutral transgene flow is closely approximated by the proportion of transgenic seed production on the landscape. However, time to equilibrium, and nonequilibrium dynamics, could vary substantially for different landscapes.

The hypothesis that gene flow is determined by total seed production on the landscape could be tested by formally parameterizing the model for several contrasting environments (*e.g.*, Northern Washington/British Columbia, the Columbia plateau, the southeastern United States, and the upper midwest). The primary requirement for adapting the model to new landscapes is a time series of GIS layers from which poplar transition rates can be derived. It is also advisable to conduct field studies of pollen, seed, and vegetative dispersal, establishment, and density-dependent mortality, all of which could vary substantially among environments. However, initial simulations could be performed with the estimates used in the current version of the model, which would yield useful information about the potential effects of the physical environment on transgene flow.

It will be difficult to apply these neutral gene flow estimates to risk assessment of transgenic poplar cultivation. It is hard to envisage a credible biological hazard posed by such a low level of gene flow, representing less than one percent of wild poplar basal area after 50 years. Non-native genes would spread to wild poplar populations at the same rates due to cultivation of conventional hybrid plantations, in the absence of transgenic cultivation. The presence of neutral transgenes would not lead to enhanced gene flow unless the added value of genetic engineering leads to an increased area of hybrid poplar cultivation. Therefore, the primary risk is the presence of the transgene in native trees. This is seen as a violation of the ‘genetic integrity’ of the species by some, because transgenes are considered unnatural due to their origin (often from sexually incompatible, evolutionary divergent organisms), and mode of transfer (Johnson and Kirby 2001; Mayer 2001; Rissler and Mellon 1996; Thompson and Strauss 2000). However, as our understanding of genomic composition of higher organisms deepens, this concept of genetic integrity is increasingly untenable. It now appears that genomes contain substantial amounts of DNA from foreign, evolutionarily divergent organisms (Kidwell and Lisch 2001), and extensive disruption, duplication and rearrangement of genetic material is the norm (Li et al 2000; Lynch and Conery 2000; Venter et al 2001). In fact, genetic engineering of poplar is most often accomplished by *Agrobacterium tumefaciens*, a native bacterium that regularly infects poplar and transfers foreign DNA to the poplar genome (Gleason 1995; Han et al 2000). However, the concept of genetic integrity transcends science, and alternative viewpoints will certainly need to be considered by policy makers in deciding an acceptable level of gene flow from hybrid poplar (Lackey 1994; Mayer 2001; Radosevich, Ghera, and Comstock 1992).

### **Insect Resistance**

Gene flow with an insect resistance transgene strongly depended both on insect pressure and the average transgenic competitive advantage. In scenarios with extensive and continuous insect pressure and a strong competitive advantage, there was a continual increase of transgenic trees through 100 years with no sign of abatement. The implication is that transgenes would eventually fully introgress into native tree populations, with some adverse ecological consequences for nontarget organisms. This

impact was substantially ameliorated for transgenics with greatly reduced fertility (0.001), but the trend was still a gradual increase in transgenics through time.

These scenarios are certainly alarming, and they highlight the importance of carefully examining the implications of deploying transgenic trees with the potential to enhance competitiveness in the wild. However, it is important to examine the assumptions underlying these insect resistance scenarios to assess the plausibility of the outcomes. One requirement for rapid and continual increase of transgenics is an insect attack that covers a substantial portion of the landscape continuously for decades. This is may be an unlikely scenario because insect attacks are often cyclical, consisting of periods of intense outbreaks alternating with periods of relative quiescence due to the effects of environmental variation and/or secondary predation/parasitism of the pest (Dwyer et al 2000; Mattson et al 1991). However, exceptions certainly exist, particularly for introduced pests with few natural checks on population growth (*e.g.*, (Louda et al 1997; McEvoy and Coombs 2000; Simberloff and Stiling 1996).

Another major requirement for a strong transgenic advantage is that the target insect must significantly compromise native tree competitiveness, so that resistance provides a significant competitive advantage to transgenics (Bartsch et al 2001; Linder and Schmitt 1994; National Research Council 2000). This is related to the first requirement for strong insect pressure, but protection from insect attack does not always translate to enhanced growth, survival or reproduction for wild trees, which may be limited by multiple factors simultaneously (Crawley 1990; Harper 1977; Parker and Kareiva 1996). Insects would have to consistently be one of the main factors limiting growth for the required level of transgenic advantage to be manifested. However, there are certainly examples in which protection from insects leads to enhanced performance in the wild (*e.g.*, (Louda 1999; Marvier and Kareiva 1999). Also, experience with introduced insects for biological control of weeds provides some striking demonstrations of potential population-limiting effects of single insect pests (*e.g.*, (Louda, Kendall, Connor, and Simberloff 1997; Simberloff and Stiling 1996). However, most attempts at biological control do not result in appreciable effects on the target plant, even though pest and target organisms are selected based on prospects for successfully reducing plant populations (Crawley 1989).

In the case of insect-resistant hybrid poplars, it is unclear what level of competitive advantage can be expected in the wild. Field trials with hybrid poplars transformed with various Bt genes have revealed growth enhancements from 9% to 23%, with substantial variation within and among experiments (Meilan, Ma, Eaton, Miller, Crockett, DiFazio, and Strauss 2000); unpublished data). However, as already discussed, it is far from clear how such data could be translated to wild populations, and it is likely that mean advantage in the wild would be substantially less. Also, one might expect that a resistance gene would carry ancillary costs that would lead to compromised performance in the absence of the selective factor (Bergelson and Purrington 1996). However, there has been little evidence of such costs in elite transgenic lines in poplar field trials thus far (Meilan, Ma, Eaton, Miller, Crockett, DiFazio, and Strauss 2000), but such effects could become apparent in more stressful wild environments, or in different genetic backgrounds as the transgene begins introgressing.

More field data are required before a definitive risk assessment for transgenic insect-resistant poplars can be undertaken. An assessment of potential gene flow will require information on the distribution and impacts of insects in wild poplar stands, as well as level of growth enhancement in wild seedlings in a variety of environments following controlled introgression of the transgene by manual backcrossing (Snow 1999). Use of sexually sterile transgenic trees would diminish the need for such studies, though a very low level of gene flow may still occur through vegetative propagation and occasional reversion to fertility. Although the simulations indicated that this would constitute a very minor portion of wild populations for at least a century, introgression into native tree populations potentiates other ecological impacts of insect-resistant transgenics, and major consideration will need to be given to the potential for evolution of resistance to the pesticidal agent, direct effects on nontarget organisms, and indirect effects on other species (James 1997; Raffa 2001).

Results of our survey of resource professionals (Appendix) provide some insight that can be applied to risk assessment of insect-resistant poplars. Most researchers who work closely with poplar plantations and/or on lands near poplar plantations indicated that insect attack is common and extensive in plantations, but there was less agreement about insect attack in wild populations of poplar. This may be because insect attack in

plantations attracts more attention because there is a direct economic impact. Also, leaf-feeding insects are more noticeable in plantation trees than in the high canopies of wild trees. However, wild poplar populations can be extremely heterogeneous both genetically and ecologically, so insect outbreaks might be less likely than in the relatively uniform plantation setting (Burdon and Walter 2001; National Research Council 2000). Two comments by entomologists are telling. For example, respondent 39 stated “There are certainly many insects associated with wild poplars, some native and some introduced. At times the populations of these insects may reach levels at which they significantly impact the survival and growth of poplars in some areas. Whether this is considered “common and extensive” damage is a matter of judgment.” Also, respondent 36 observed, “A different complex occurs in wild vs plantation poplars. Damage in wild poplars is episodic...(damage is) more common and more extensive in plantations.”

Another interesting survey result is that so few of the respondents thought that a 10-20% growth advantage in the wild due to insect resistance would make poplars more difficult to control (none thought that this was ‘very likely’). In contrast, the model results indicated that a 10-20% growth advantage was nearly enough to ensure a gradual introgression of transgenes into native tree populations. Many respondents believed that poplar would be easy to control in managed settings, and insect resistance would not change this. They apparently did not consider introgression into native populations as a viable risk.

## **Herbicide Tolerance**

Impacts of herbicide tolerance are likely to be more agronomic than ecological. Herbicide tolerance provides no benefit in environments where the herbicide is not used (Strauss et al 1997; Warwick 1991). In fact, herbicide resistance could carry a fitness cost (Coghlan 1999; Purrington 2000; Warwick 1991), so spread could be impeded in the wild (but see (Purrington and Bergelson 1997) and (Bergelson et al 1998)). Thus far, few pleiotropic effects have been observed in extensive field testing of poplars carrying the glyphosate resistance trait (Meilan et al. 2000).

Some key agronomic risks are spread of resistance to weeds through gene flow with wild relatives, shifts in weed populations to resistant individuals and species due to

overuse of a particular herbicide, and creation of new weed problems where crops with the same resistance gene are rotated (Burnside 1996; Duke 1999).

Glyphosate tolerance resulted in little change in overall transgene flow compared to neutral transgenics across a broad range of parameter values. This is due in part to the relative lack of habitat availability in environments where glyphosate is used, and therefore where the transgenic advantage was manifested. Glyphosate tolerance did result in an increase in agricultural establishment, though overall rates of agricultural establishment remained quite low. Maximum observed rates in the test landscape were for the scenario with an establishment rate of 0.01 (1% of field area converts to poplar annually). This is very obviously an unrealistic rate of poplar establishment in the study area, as a cursory survey of fields would illustrate. It is extremely rare to find poplar volunteers in agricultural fields of any kind in this environment. The baseline establishment rate of 0.0001 is probably more realistic, and this resulted in agricultural establishment rates of less than 0.01%, even without engineered sterility.

A major determinant of the impact of gene flow from herbicide-resistant transgenic plantations is the availability and distribution of sites where poplars are capable of establishing, and where glyphosate is the primary means of weed control. Such sites are likely to be highly restricted in the modeled lower Columbia River landscape, where agricultural fields are typically tilled annually, pastures are heavily grazed, and weed control is typically performed with a 'tank mix' containing a variety of herbicides to which poplar is susceptible (Appendix;(Burrill et al 1992; National Agricultural Statistics Service 1999; Strauss, Knowe, and Jenkins 1997). Candidate sites include field edges, roadsides, drainage ditches and lowland tree plantations (Appendix). Such sites constitute a small proportion of the landscape, but because of their large stature and ability to sprout, established poplars could cause a significant inconvenience for landowners and managers. However, only 18% of the survey respondents considered poplar to be an invasive weed (Appendix), suggesting that the problem will be minor.

Potential impacts of herbicide resistance transgene flow will vary substantially among settings. The amplitude of available habitat increases from south to north and east to west in the Pacific northwest, with more mesic conditions favoring establishment of poplars at increasing distances from riparian areas (Appendix;(DeBell 1990;

U.S. Environmental Protection Agency 1999; Zasada et al 1981). Also, there are locations where glyphosate is virtually the sole option for chemical weed control because it is a relatively benign herbicide with low mammalian toxicity and rapid immobilization and degradation (Environmental Protection Agency 1993). For example, in Canadian forests glyphosate is nearly the only herbicide used in aerial applications (Environment Canada 2001). In combination with the broad habitats of poplars in British Columbia, the potential for negative impacts of glyphosate tolerance transgene flow are considerable. In contrast, poplars are virtually restricted to areas with high water tables (*e.g.*, streamsides, irrigation ditches, springs) in the high desert of eastern Oregon and Washington, and glyphosate is typically used in conjunction with herbicides such as triclopyr, 2,4-Dichlorophenoxyacetic acid, sulfometuron methyl, and imazapyr (Appendix (Strauss, Knowe, and Jenkins 1997)). Impacts of herbicide-resistant poplars should be comparatively small in these areas.

An accurate assessment of frequency of establishment in managed areas and patterns of glyphosate usage would allow more precise calculation of expected economic impacts from glyphosate-resistant poplars. With currently available information we can state that impacts will likely be small in Oregon and southern Washington, but potentially much greater in northern Washington and Canada.

## Chapter 5 Conclusions

Biotechnology presents both substantial opportunities and challenges to forestry professionals. Gene transfer will soon be applied to a wide variety of trees, raising the possibility that plantations of the future may be tailored to enhance production of lumber, biofuels, readily-extracted pulp, or perform other environmental services. However, there is also substantial concern that this power could produce unforeseen negative consequences for managed and natural ecosystems. Regulators and scientists are charged with assessing the risks of these consequences, but traditional risk assessment methods are difficult to apply in forest systems. Potential impacts would occur over large spatial and temporal scales, precluding direct observation and experimentation.

We have developed a new spatial simulation model, STEVE, to facilitate the study of transgene flow and risk assessment for transgenic tree plantations. The model integrates information about the processes involved in gene flow, and allows simulation of complex scenarios involving specific traits and planting designs. However, the accuracy of model predictions depends on the veracity of the underlying assumptions and/or parameter estimates. Therefore, it is important to apply the model cautiously and to examine model predictions in the light of independent knowledge. I will outline uses of the model that I deem appropriate given the current state of knowledge and development, and highlight some areas in need of additional work to further refine model estimates.

One appropriate application of the STEVE model is to use sensitivity analyses to identify parameters with the largest influence on model outcomes, as described in Chapter 2. Parameters can be ranked in order of strength of effect across a biologically reasonable range of values. Confidence in significance of a parameter is increased if effects occur across a broad range of model conditions. This was the case for transgenic competitiveness, transgenic fertility, disturbance rates, and basal area of plantations (rotation length), each of which had significant effects on transgene flow under all tested conditions. Factors that had significant effects under only a subset of conditions were distant pollination, distant seed dispersal, vegetative establishment, and vegetative dispersal.

Future research efforts aimed at transgenic risk assessment for poplar should first focus on estimating rates of landscape disturbance and fitness effects of transgenes, rather than fertility or silvicultural factors. Fertility of transgenic trees is likely to be too low to have a strong effect on gene flow, at least for the first generation of commercial transgenic trees. This is mainly because hybrid clones currently in use have innately low fertility compared to wild trees (Chapter 3). Also, for regulatory simplicity and transgene stability, most commercial transgenic trees will have only a single copy of the transgene insert, resulting in a 50% reduction in the frequency of transgenic progeny compared to modeled conditions. Finally, genetically engineered fertility reduction is likely to be required for near-term commercial uses of transgenic trees. In the case of rotation length, it is reasonable to assume that rotations will not exceed 12 years for the foreseeable future because of market considerations and regulations, so this factor is essentially fixed. Thus, disturbance regime and transgenic competitiveness, rather than fertility or rotation length, are the key factors with strong effects on gene flow that merit further consideration for refining model estimates of gene flow.

I recommend focusing initial research efforts on further characterization of the disturbance regime, rather than on competitive effects of transgenes. One reason is that the disturbance regime had a stronger influence on transgene flow in scenarios involving reduced fertility, while effects of transgenic competitiveness were diminished for those scenarios. Also, studies of transgenic competitive advantage would be technically and conceptually complex, requiring examination of a large number of genotypes and environments (Crawley et al. 2001). Moreover, there are currently few transgenes that would be likely to enhance competitiveness in wild settings, and these can often be readily identified *a priori* (Strauss et al. 2001a), so extensive field tests to assess competitive advantage will not always be warranted (Kareiva et al. 1996). In contrast, disturbance regime is important for risk assessment for all transgenic traits, and could be accomplished using readily-available air photos and standard GIS techniques (Allen 1999). Research resources would thus be most efficiently allocated by initially focusing on the disturbance regime.

It may also be desirable to further characterize long-distance dispersal of pollen and seeds, and vegetative establishment, all of which were increasingly important as fertility

declined. However, studies of long distance dispersal are extremely difficult and costly using currently available technology (Cain *et al.* 2000). A possible solution is to allow large field trials or commercial cultivation of transgenic trees with a low-risk, selectively neutral trait (*e.g.*, modified lignin), that also has an easily-assayed marker phenotype such as Green Fluorescent Protein (Stewart 1996). If the transgenic phenotype could be readily identified in progeny, this would greatly facilitate studies of long-distance gene flow and establishment.

Findings with the STEVE model are potentially useful for assessing risks of transgenic trees, even without further modification or research. Once endpoints of concern are identified, the model could be used to perform virtual dose-response experiments that could aid in assessing outcomes across a wide range of scenarios. For example, if extensive introgression of Bt transgenes in native populations is identified as an endpoint of concern, STEVE could be used to explore conditions that result in a continual increase in transgene frequency. In our sensitivity analyses of transgenic competitive advantage, we identified a threshold value that resulted in an upward trend in transgene frequency through time. The challenge is to determine how accurately model conditions represent real-world conditions. The model provides clear, testable hypotheses that can guide further research or adaptive management programs. In the case of transgenic competitiveness, the model could be calibrated by comparing model predictions to actual growth and survival of transgenic trees in plots that mimic wild conditions. In the case of Bt trees, the model could be used to explore a wide range of conditions and identify thresholds of insect pressure and competitive advantage required for significant introgression. These estimates could then be used as benchmarks in the interpretation of field results.

Finally, the model can help guide the intensity of monitoring efforts. For example, STEVE simulations indicated that instability in transgenic sterility was only important for gene flow if rates of reversion to fertility were quite high, and reversion was persistent between years (Chapter 2). This suggests that screening trials aimed at assessing stability of transgenic sterility could be of moderate size and duration, because estimation of low-frequency events would be of little value for minimizing transgene flow.

Validation of model predictions is essential if STEVE is to be used for risk assessment and monitoring. Our analyses suggested that the model is conservative, consistently overpredicting transgene flow. In particular, modeled seed dispersal and establishment of progeny of plantation trees were each well above rates observed in field studies. A number of conservative assumptions contributed to these overestimates:

- *Changes in habitats delineated from air photos represent establishment and mortality of poplar.* In fact, many of the apparent changes were due to inaccuracies in the delineating and digitizing process, so modeled transition rates were likely higher than actual transition rates, so more establishment opportunities were created for transgenic seedlings. Further research is required to gauge the magnitude of this error.
- *Local pollen availability does not limit seed production.* Even in cases where there are no fertile local males, we allow full seed production for female trees. There is no evidence for pollen limitation of *Populus* in nature (Eckenwalder 1996; unpublished data). However, based on our studies of long-distance gene flow in native poplar populations (unpublished), it is very likely that background (long-distance) pollination would be enhanced where local pollen production is limited. This assumption results in inflated transgenic seed production under low fertility levels, because 50% of seeds are sired by local trees in our model, even if pollen production is extremely low.
- *Fertility of conventional hybrid trees is the same as fertility of wild trees.* This is untrue for most hybrid and wild trees that have been examined. Wild female trees typically produce more than twice as many viable seeds per catkin as hybrid trees, for example (Chapter 3; personal observation). This is likely to cause an overestimate of seed production for transgenic males (which tend to heavily pollinate nearby conventional plantation females in the model), particularly in scenarios with low transgenic fertility.
- *The transgene does not segregate.* We assumed that the transgene could be present in a large number of unlinked copies, and therefore ignored segregation in progeny of transgenics. In reality, most commercial transgenic plants will

contain only a single transgene, so this assumption results in overestimating gene flow by 100% each generation.

- *Seeds do not limit establishment.* Establishment is insensitive to the number of seeds produced in the direct vicinity of a new establishment site. If no seeds are produced in the neighborhood, a full cohort can still become established, and genotypes are determined by representation in the landscape seed pool. This assumes abundant long-distance seed dispersal covering the entire landscape, which is not realistic for poplar (personal observation). The net effect is an overestimation of long-distance transgene movement.
- *Long-distance seed establishment accounts for 10% of all new cohorts.* This is related to the previous assumption. As a result, transgenic seedlings were initially present in all new cohorts for the commercial cultivation scenario. Transgenes that confer a large selective advantage can rapidly introgress throughout wild populations due to this assumption, because all establishment sites are eventually captured by transgenic trees in these scenarios. In reality, many sites are likely to be colonized overwhelmingly by locally produced seeds, particularly when the local seed source is large.
- *Vegetative establishment can account for up to 10% of all new cohorts.* If poplar trees are present within the dispersal neighborhood, vegetative propagules of each genotype may become established proportional to basal area of each genotype. Frequency of vegetative establishment is unknown, but we estimated 10% based on the frequency of identical ramets in a number of different populations. This is almost certainly an overestimate because poplar clones expand over time by local root sprouting and dispersal, and the clones examined may have been quite old.
- *Density-dependent mortality and competition are deterministic.* There is no mechanism in the STEVE model for stochasticity to play a role in density-dependent mortality. Entire cohorts may be removed by stochastic disturbance, but mortality within a cohort is determined entirely by the initial starting density of conventional and transgenic seedlings, and the transgenic competitive differential. As a result, spread of transgenic trees is overestimated for scenarios with a strong transgenic competitive advantage.

We made these assumptions with the intention of providing upper estimates of potential transgene flow from plantations. The net effect of multiple, independent overestimates is multiplicative, so gene flow estimates could be orders of magnitude too high for some scenarios (particularly those involving a transgenic competitive advantage). Ultimately, only monitoring of large, long-term field trials or commercial plantations will provide the data needed to produce realistic estimates of transgene flow. Model estimates can be refined as information about the relevant processes continues to accumulate. However, current gene flow estimates may be used as a conservative starting point in assessing hazards posed by cultivation of transgenic trees.

In conclusion, the STEVE model can play a key role in practical applications such as risk assessment for transgenic organisms, where it provides bounds for reasonable levels of expected impacts, and informs monitoring programs for assessing transgene effects. The model can also aid in basic scientific inquiries about ecological and genetic factors controlling tree demographics on a landscape scale. The model can also be readily adapted for diverse landscapes, and expanded to simulate the dynamics of species other than poplar. The program is available, together with a user manual and source code, at: [http://www.fsl.orst.edu/tgerc/STEVE\\_model/](http://www.fsl.orst.edu/tgerc/STEVE_model/).

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## **Appendix**

## Appendix Survey of Resource Professionals

We conducted a web and mail survey of a wide variety of professionals with expertise in areas that could potentially be impacted by transgenic poplar. We sought professionals, including academic scientists, in four main areas: agronomy, forestry, conservation, and vegetation control. Names were obtained through agricultural and forestry extension mailing lists, a literature review, and personal contacts provided by academic and professional organizations. We sought people in the following fields:

- agronomists with knowledge of weed control for various crops and nurseries in the vicinity of cottonwood plantations;
- foresters with experience in hybrid poplar cultivation, Christmas tree farming, and tree nurseries;
- conservationists with experience in riparian areas and upland forests in areas of cottonwood cultivation;
- vegetation control specialists, including reforestation specialists and spray contractors; and
- entomologists with knowledge of pests of poplar.

We contacted professionals by electronic mail and directed them to the URL for the survey ([http://www.fsl.orst.edu/tgerc/poplar\\_survey.htm](http://www.fsl.orst.edu/tgerc/poplar_survey.htm)). We also provided the option of responding by fax or conventional mail.

We contacted approximately 200 people, approximately 100 of whom were forestry professionals in academia and industry, and the rest equally divided between agronomists, conservationists, and vegetation control specialists. The survey was introduced by a cover letter (<http://www.fsl.orst.edu/tgerc/Survcovlet.htm>) to orient respondents to the nature of the issues, and to ensure informed consent of the respondents. Forty-eight people responded, 41 to the web survey and 7 by conventional mail. The vast majority of respondents (76%) had strong ties to forestry, which may indicate that many respondents had a direct interest in the topic of the survey. However, 56% of respondents were from academia, and most professed only a 'basic awareness' of genetic engineering. Therefore, it is fair to assume that most respondents had no personal

interest in the fate of forest biotechnology. Also, a number of respondents had expertise in ecology, environmental management, and entomology, so a broad array of expertise and backgrounds were represented.

We asked questions in four main areas: personal information, current status of poplar, anticipated effects of introduced traits, and attitudes about biotechnology. The “Personal Information” section was intended to gather background information about the respondent and their area of expertise. The “Current Status of Poplar” section provided information about the current invasiveness of poplar and its management in different settings. The “Anticipated Effects of Introduced Traits” section obtained opinions about likely impacts of trees engineered to be reproductively sterile, resistant to glyphosate-based herbicides, or resistant to defoliating insects. Responses to the “Attitudes about Genetic Engineering” section helped us to evaluate how different perspectives about biotechnology might have influenced responses. A major finding is that many respondents believed that herbicide resistance and insect resistance would likely present minor and manageable environmental and agronomic difficulties, particularly if the transgenic trees are reproductively sterile. However, there was a great deal of uncertainty and diversity of opinion. Respondents could be divided roughly into two groups: those who thought that GE poplars raised significant ethical issues and uncertain risks, and those who were relatively comfortable with the technology and associated risks.

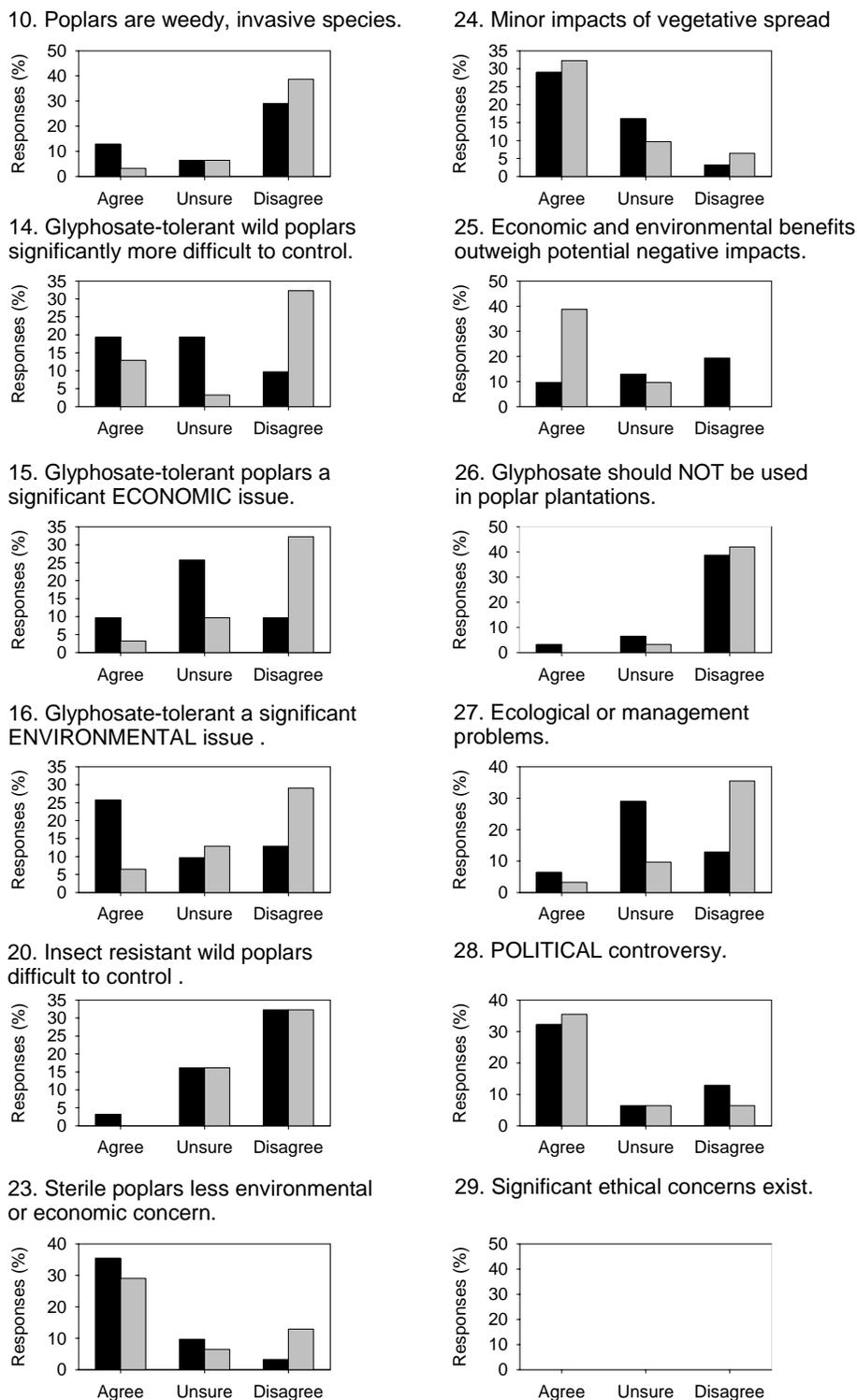
Other highlights:

- 65% did not consider poplars weedy, invasive and difficult to control, while 19% believed poplars are invasive. This discrepancy was partly due to regional differences (invasiveness increases east to west and south to north), and differences in orientation of respondent (those who focused on ease of control didn't consider cottonwood problematic).
- In some circumstances, glyphosate can be the primary or sole herbicide for controlling weeds in all settings we listed. However, the consensus was that this is the exception rather than the rule, and is highly situation-specific. One exception might be Canadian forests, where glyphosate is often the only herbicide

allowed. Another was riparian areas where glyphosate is often the only herbicide used due to its low toxicity to aquatic life (in the Rodeo<sup>®</sup> formulation).

- Although glyphosate may not be the PREFERRED method of controlling poplar, 42% of respondents indicated that it is still a major means of controlling wild poplars; mechanical control is also quite common (58% of respondents, including comments)
- Opinion was divided on whether glyphosate-tolerant poplars would be more difficult to control: 35% yes, 38% no. Again, regional differences account for some of this.
- Only 12.5% believed RR poplars would present serious economic problems, while 48% don't see significant economic problems. In contrast, 33.3% expected an environmental impact, while 37.5% don't see serious environmental issues. The difference seems to be due to concern over increased use of more toxic alternatives to glyphosate to control cottonwood escapes.
- 48% believed that insect damage is common in poplar plantations, 17% do not. In contrast, only 31% believe insect damage is common in wild populations, and 29% do not.. Annual variations were cited for both, but perhaps more important in wild populations.
- 19% believed Bt is a major means for controlling defoliating insects of poplar; while 35% professed no knowledge. Bt spray is mostly used for control of lepidopterans (forest tent caterpillar), so this is not relevant to *Chysomelid*-specific Bt toxins currently in use in poplar.
- None of the respondents believed it was 'very likely' that a 10 to 20% growth enhancement due to insect resistance in the wild would make poplars significantly more difficult to control; only 8% considered it likely, and 69% considered it unlikely or very unlikely. There was more consensus here than on most of the survey.
- Many respondents believed that use of sexually fertile trees is likely to cause only minor impacts on agriculture, forestry, and the environment. Only 19% disagreed, and 44% agreed or strongly agreed that impacts are likely to be minor

- 65% thought sterility added to environmental safety; the 12.5% who disagreed were mainly concerned about vegetative spread. It appears that the one person who strongly disagreed (respondent 25) made an error, judging by his answers to the other questions.
- Similarly, 58% agreed or strongly agreed that vegetative spread would be minor and manageable. only 10% had significant concerns here.
- Most believed economic and environmental benefits from transgenic poplars outweigh possible concerns: 44% agree/strongly agree; 29% unsure; 17% disagree/strongly disagree
- Most respondents (83%) disagreed or strongly disagreed with the statement that glyphosate shouldn't be used in plantations; only one agreed, and one strongly agreed. The person who strongly agreed stated that it shouldn't be used in plantations because it wasn't labeled for such, and it would kill the trees . The person who agreed thought mechanical control was best (an entomologist)
- There were no strong opinions that GM poplars would cause unanticipated and significant problems in plantations: only 6.25% agreed with this statement, and 48% disagreed or strongly disagreed; several expressed the caveat that this is contingent on the use of sterile trees. 46% were unsure or failed to respond.
- In contrast, 67% believed political problems would result, and only 12.5% disagreed with this statement.
- The respondents were evenly split on the question of whether transgenic poplars raised significant ethical issues, though nearly half of respondents were unsure. This division differentiates responses to other questions: those who did not see substantial ethical issues with genetic engineering also tended to see few ecological, economic, or management problems, while those with ethical concerns were less sanguine about possible problems in other areas (Figure A.1).



**Figure A.1** Responses to selected questions, separated by response to question 29. Dark shaded bars are responses of those who believed genetically engineered poplars raise substantial ethical concerns; gray bars are for those who did not have ethical concerns.

Detailed responses to each question are provided below. Included are the percentage of respondents for each broad category of answer, as well as additional information/comments, identified by the number of the respondent. Therefore, it is possible to trace comments of individual respondents by viewing the same bold number followed by a colon, following each question. Responses have not been edited for spelling or grammar.

### **Question 1**

Please briefly describe your occupation and education.

Forestry/Agronomy. 27.08%  
 Forest Management. 10.42%  
 Poplar Management. 16.67%  
 Forestry/Agronomy Extension. 22.92%  
 Ecology. 8.33%  
 Environmental Management. 6.25%  
 Entomology. 8.33%

- 1:** Professor, Forest Science
- 2:** Professor of Forestry
- 3:** extension/research
- 4:** Prof. of Forest Genetics; Recd Dec. 13, 1999
- 5:** Forestry
- 6:** Investigator, WSDA
- 7:** Extension agent, Aurora, OR
- 8:** Forester for a small, family-owned private forest products manufacturing company.
- 9:** Retired forester Willamette National Forest
- 10:** Intensive Management Forester (Reforestation Forester)
- 11:** Extension Forester, Linn and Benton Counties
- 12:** Forestry Extension Agent, Coos County. Provides forestry education to the public
- 13:** Watershed Management Extension Agent for OSU. Work with watershed councils and landowners in Western Oregon to implement watershed enhancement projects.
- 14:** Professor of Forest Science and Interim Dean/Director, College of Forestry/Forest Research Laboratory, Oregon State University
- 15:** Public educator and advisor with Washington State University Cooperative Extension in Southwest Washington, Cowlitz and Wahkiakum counties, also interim State Community Horticulture and Master Gardener Coordinator. Prior career work at the Washington State Department of Natural Resources, Webster Forest Nursery as an assistant grower.
- 16:** Oregon State University Extension Agent - Crook Co. 13 years Work with private land owners, agricultural producers, and general public in the areas of range, livestock and natural resource issues.

- 17:** OSU Extension agent with applied research and educational responsibilities for agronomic crops for Marion and Clackamas county. I have the rank of professor in the Dept. of Crop and Soil Science at OSU. I have been with OSU for 26 years.
- 18:** Research Rangeland Ecologist, USGS, FRESA with a research emphasis on revegetation and restoration of native plant ecosystems in the Intermountain West.
- 19:** Professor of Weed Science, Editor, Weed Science
- 20:** Consultant in the field of short rotation woody crops, in particular hybrid poplar.
- 21:** Professor of Horticulture specializing in research on pears, apples, and cherries. Primary research is on rootstocks, training systems, and fruit quality.
- 22:** Wetlands ecologist with Oregon Natural Heritage Program, Nature Conservancy of Oregon
- 23:** Extension Forester, OSU Extension Service
- 24:** Retired professor of weed science.
- 25:** Research and development
- 26:** Assoc Prof, Forest Science
- 27:** Research Entomologist and Adjunct Assoc. Prof. Entomology. My research interests involve the use of microorganisms for management of insect pests.
- 28:** County Extension Agent, field faculty Oregon State University Provide public with research based information
- 29:** Lane County Extension Agent
- 30:** Agricultural Research Assistant --- primary focus is potatoes and sugarbeets
- 31:** I am a Survey Plant Pathologist with the Oregon Dept. of Agriculture. My main focus is the Imported Timber Health Program, although I also participate in the review of notifications for genetically modified organisms.
- 32:** Botanist, Plant Conservation Biologist I work is a regulatory and research program to develop methods for restoring and conserving endangered plant species.
- 33:** Natural Areas Ecologist. I conduct monitoring of rare plants and ecosystems, manage weed control and restoration projects.
- 34:** Manage poplar genetic improvement program-breeding, clone testing, scale-up, deployment. Manage productivity research.
- 35:** Academic teaching, research & consulting on agroforestry issues and riparian landscape ecology
- 36:** Professor of Forest Entomology
- 37:** Research Forester, specializing in reforestation, weed ecology and vegetation management
- 38:** Stewardship Forester, I work with NIPF landowners on giving advice for managing their land, I also manage three natural areas.
- 39:** Associate Professor, Forest Entomology, Integrated Forest Protection
- 40:** Professor of ecology and silviculture
- 41:** Extension forester-OSU
- 42:** Fiber farm manager for Boise Cascade Corporation
- 43:** Research Technician for Boise Cascade's Cottonwood Fiber Farm in Wallula, WA.
- 44:** Farm superintendent responsible for the harvesting, site preparation, establishment, cultivation, and maintenance of hybrid poplar plantations.
- 45:** Research entomologist, study the use of insect pathogens for control of agriculturally important insect pests.

**46:** Poplar Geneticist for Boise Cascade Corporation (translation: I run the cottonwood breeding program)

**47:** Research Supervisor for a forest products company. Coordinate the research activities for a company unit that is focused upon short rotation intensive culture of hybrid cottonwoods.

**48:** nurseryman

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### **Question 2**

Current employer (circle one).

- A. Academic 56.25%
  - B. Government agency. 16.67%
  - C. Small private industry. 6.25%
  - D. Corporation. 10.42%
  - E. Non profit organization. 2.08%
  - F. Self-employed. 6.25%
- 

### **Question 3**

What are your main areas of expertise and experience? (Circle up to three).

- A. Agronomy/farming 37.50%
- B. Forestry 62.50%
- C. Ecology/environmental management 33.33%
- D. Vegetation control 29.17%
- E. Insect pest control 14.58%
- F. Other (please specify) 35.42%

**4:** plant genetics **7:** stress physiology of woody plants **9:** community development **13:** hydrology **14:** Academic Administration **15:** Horticulture **16:** water quality, ranching **20:** Poplar farming (SRIC) **21:** plant physiology **25:** Integration of ideas **27:** microbiology **28:** Education **31:** Plant Pathology (emphasis on Forest Pathology), Molecular Biology, and Plant Breeding. **33:** rare plant management **46:** tree breeding **47:** Forest Genetics **48:** nursery propagation

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**Question 4**

In which settings do you have the most experience? (Circle all that apply).

- A. Agricultural fields 41.67%
- B. Conifer plantations 43.75%
- C. Tree nurseries 22.92%
- D. Orchards/vineyards 16.67%
- E. Poplar plantations 50.00%
- F. Riparian Areas 27.08%
- G. Upland (conifer) forests 39.58%
- H. Roadsides/rights of way 10.42%
- I. Other (please specify) 33.33%

**7:** Landscapes: commercial and residential **14:** Behind a Desk! **15:** nursery production and horticulture **16:** rangelands **18:** semi-arid rangelands **22:** wetlands **25:** Wilderness, revegetation **26:** Prairies **27:** northern hardwood forests **31:** urban environments, hardwood forests, mixed deciduous/coniferous forests **32:** Natural habitats **33:** shrub-steppe, natural areas, shrub-steppe rangeland. Also, in the natural areas field, glyphosate is one of the most preferred herbicides, when herbicides are necessary. This is true in all types of ecosystems, riparian, wetland, shrub-steppe, forest, prairie, etc. **34:** home gardeners **35:** wetlands **46:** greenhouse **47:** home use to control weeds in and around gardens

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**Question 5**

In which geographic area(s) do you have substantial experience? (Circle all that apply).

- A. Western Oregon 58.33%
- B. Western Washington 27.08%
- C. East of the Cascade Mountains 54.17%
- D. Other (please specify) 27.08%

**2:** Northeast U.S., SE Asia **12:** northern California **14:** Southeastern USA (Kentucky, Mississippi, Louisiana) **19:** Rocky Mountain West - Colorado **20:** Southwestern B.C. **25:** California (central, southern, coastal, mountains) Louisiana, Brazil (central, south central, Amazon), Paraguay **27:** eastern U.S. **31:** upper Midwest **34:** North central Idaho, Minnesota **35:** Rocky Mountain States **36:** Great Lakes Region **42:** south, midwest, New England **47:** Boreal regions

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**Question 6**

Highest degree:

- A. High School diploma 0
- B. Bachelor degree (list major) 18.75%
- C. Post-graduate degree, not doctorate(list major) 35.42%
- D. Doctorate (list major) 41.67%

Majors:

Weed Science 4.17%  
 Forestry 29.17%  
 Genetics 8.33%  
 Biology 4.17%  
 Horticulture 12.50%  
 Range Management 2.08%  
 Botany 8.33%  
 Agronomy/Crop Science 6.25%  
 Entomology 10.42%  
 Ecology 8.33%  
 Environmental Management 4.17%  
 Engineering 2.08%

**1:**PhDmaj: Weed Science **2:**PhDmaj: Forestry **3:**PGMajor: Forestry/Business **4:**PhDmaj: Genetics **5:**PhDmaj: Forest Tree Physiology **6:**BMajor: Biology/Chemistry :PGMajor: Public Administration **7:**BMajor: pomology :PGMajor: Masters-Horticulture :PhDmaj: Horticulture **8:**BMajor: Forest Mgt; Range Mgt **9:**BMajor: Forestry **10:**BMajor: Foresstry **11:**BMajor: horticulture :PGMajor: forest ecology :PhDmaj: Forest ecology **12:**PGMajor: MS Silviculture **13:**PGMajor: M.S. Bioresource Engineering **14:**PhDmaj: Genetics **15:**BMajor: Horticulture :PGMajor: some coursework **16:**BMajor: rangeland resources :PGMajor: Range Management **17:**PGMajor: MS in Crop Science **18:**PhDmaj: Botany **19:**PhDmaj: Agronomy/Agricultural Chemistry **20:**PGMajor: M.Sc. Forestry Technique & Silviculture **21:**PhDmaj: Horticulture **22:**PGMajor: Botany **23:**PGMajor: Forest Entomology **24:**PhDmaj: Weed Science in Farm Crops **25:**BMajor: Mathematics :PGMajor: Horticulture :PhDmaj: Plant physiology **26:**PhDmaj: Ecology **27:**PhDmaj: Entomology and parasitology **28:**BMajor: Animal Science and Environemntal Education :PGMajor: not finished in Resource Recreation Mgmt **29:**PGMajor: forest products **30:**BMajor: Crop & Soil Science **31:**PhDmaj: Botany & Plant Pathology **32:**PGMajor: Plant ecology **33:**PGMajor: Natural Ecosystem Management **34:**PGMajor: Silviculture and genetics **35:**PhDmaj: Botany **36:**PhDmaj: entomology **37:**PGMajor: Forest Ecology/silviculture **38:**BMajor: Forest Resource mgt. **39:**PhDmaj: Entomology **40:**PhDmaj: Forest ecology **41:**PGMajor: forestry **42:**PGMajor: Silviculture **43:**BMajor: Forest Resource Management **44:**PGMajor: Engineering Science **45:**PhDmaj: Entomology **46:**PhDmaj: Plant Breeding and Genetics-Forestry **47:**PGMajor: Forest Genetics **48:**BMajor: horticulture

**Question 7**

How familiar are you with genetic engineering?

- A. Unfamiliar 0
- B. Basic awareness 52.08%
- C. Considerable understanding 33.33%
- D. Technical comprehension 14.58%

**Question 8**

Can you distinguish poplars (including cottonwoods and aspens) from other trees?

- A. Usually 97.92%
- B. Sometimes 2.08%
- C. Never 0
- D. Don't know 0

**Question 9**

Please circle settings where you commonly observe young poplar trees GROWING NATURALLY (*i.e.*, not planted).

- A. Agricultural fields 39.58%
- B. Conifer plantations 18.75%
- C. Tree nurseries 8.33%
- D. Orchards/vineyards 4.17%
- E. Poplar plantations 37.50%
- F. Riparian Areas 87.50%
- G. Upland forests 29.17%
- H. Roadsides/rights of way 47.92%
- I. Other (please specify) 29.17%

**1:** drainage areas; **2:** clearcuts in north **18:** Aspen Groves **21:** residential **22:** wetlands, seepage areas **27:** abandoned fields, disturbed sites **28:** windbreaks **30:** homestead areas **31:** near wetlands, poplar groves associated with farmhouses, mixed deciduous and coniferous forests (*e.g.*, northern Minnesota) **33:** Never. **34:** Areas adjacent to ag. fields(hedge rows in MN) **35:** Lakeshores **45:** springs **47:** Irrigation ditch banks, areas of disturbed soil such as around new house construction, gardens

## Comments:

- 1:** Cherry orchards near riparian areas; drainage areas of agriculture fields; in upland riparian areas
- 2:** Increasingly in uplands in western Washington, BC as you head north
- 4:** Naturally = spontaneously
- 12:** Familiar with the Fort James lower Columbia plantations. Worked for the company that developed that technology in the early 70's
- 14:** *Populus trichocarpa* does not seem to be as aggressive a colonizer in W. Oregon as it is in E. Oregon/WA. Nor does it seem to be as aggressive, in general, as *P. deltoides* in eastern USA.
- 15:** depends on the site they are growing
- 18:** The poplars in the Agricultural fields are normally in riparian areas.
- 20:** In poplar plantations, seed-in usually occurs in the rows, depending on the cultivation and herbicide regime used. The trees are always suppressed by the much faster growing hybrids, which started from cuttings rather than seed as do the naturals.
- 25:** By poplar here I am including cottonwoods which routinely emerge in our production fields, but fail to survive a single season due to cultivation, etc.
- 27:** different populus species are indicative of different sites and conditions
- 31:** Steve, this is a difficult question to answer as you've formatted it. It could be argued that poplars can be found growing naturally in any situation because of their suckering capabilities (*e.g.*, there would be natural suckers in poplar plantations).
- 33:** By poplar, I assume you mean something other than aspen or cottonwood, which are the only native *Populus* in the areas I work in. Therefore, all "poplar" I see have been planted.
- 34:** Successful establishment is not an every year event on the eastside. Ideal conditions must exist for establishment. Those seedlings that germinate in plantations are at a competitive disadvantage and quickly become suppressed and die.
- 37:** *Populus Trichocarpa* is generally much more common outside of classic riparian zones in Western WA particularly north of Castle Rock. I typically find it more frequently lower elevation west side cascade sites
- 40:** rare in W Oregon cascade forests, common in lower riparian areas. Common in hills N of Seattle
- 43:** It is rare to find a poplar growing "wild" away from a water source east of the Cascade Range.
- 47:** East of the Cascades, habitat for poplar seedling establishment is limited to riparian areas (which are themselves not plentiful, irrigation ditchbanks, agricultural fields, and other areas with disturbed soil. Riparian zones in east of the Cascades also have a rather short time frame, usually following spring floods, where conditions are suitable for seedling establishment. Such areas dry out quickly, and competition from herbaceous plants is significant
-

**Question 10**

Poplars are weedy, invasive species that are often difficult to control.

- A. Strongly Agree 2.08%
- B. Agree 16.67%
- C. Unsure/No Response 16.67%
- D. Disagree 50.00%
- E. Strongly disagree 14.58%

Comments:

**4:** West of the Cascades: Their demand for water rules them out on many sites; Alder is more invasive on more sites; Truly invasive species of riparian zone: scotch broom, Himalayan Blackberry, giant knotweed, clematis, Buddleja

**6:** 1-1.5% qt garlon 4 in spring time pre-bud break, very effective

**9:** Cottonwoods are invasive around sewer lines. In other areas they can be valuable.

**12:** Poplars are no more invasive than any other species which produces small windborn seed. ie western hemlock or alder. Technology exists to control poplar effectiveny by both mechanical and chemical means. The populus genus is listed as easily controled by herbicides containing glyphosate, triclopyr, 24-D, and picloram

**14:** See above!

**16:** Only problem area that I have experienced is when people plant aspen or cottonwood too close to septic tank or drain field.

**20:** Somewhat disagree because it depends what environment the seed-in occurs in. For example, seed-in in a bare-root tree nursery poses problems because control methods usually impact the nursery crop as well. In a container seedling nursery (in greenhouse), seed-in of cottonwood occurs as well. Incoming weeds are usually removed by hand and chemical control is hardly ever used for this, so it is a non-issue. The cottonwood ghets removed at the same time the dandelion is removed. In farmer's fields the seed-in usually occurs before the final site preparation for other crops, destroying seeded-in cottonwoods (if any). In conifer plantations control is somewhat more difficult. There usually is not a whole lot of exposed mineral soil in the plantation itself and seed-in is not an issue. I think most forest seed-in happens along exposed roadsides and areas that have been disturbed by harvesting equipment. There are herbicides available to control cottonwoods while not damaging conifers.

**27:** Populus spp. serve to stabilize and improve disturbed sites (pioneer) for other species that later dominate (succession).

**31:** I agree depending on the situation. The main problem is their suckering capability(ies). Suckers can be a fairly severe problem in "controlled growth" areas, e.g., urban landscapes. It can also cause difficulties in plantation settings, depending on the management strategies. I suppose there is the potential for environmental problems in specific environments, e.g., areas where native habitat restoration projects are underway.

**32:** I work in natural systems where native poplars can be important component--they are not viewed as weeds in this setting.

**35:** unwanted invasions are relatively easy to control

**37:** MOre so in WA than OR of CA. The invasiveness is tied to how the sites and when the sites are harvested

**42:** Rarely invade anywhere other than riparian areas

**47:** Poplar seedlings established in areas where they are unwanted are fairly easy to control, with various chemical and mechanical means. Once the trees become established and are larger than a young seedling, control can be more difficult. However, I am not aware of a situation where poplar seedlings have ever become a weed problem.

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### **Question 11**

Please indicate settings where you know that glyphosate-based herbicides (*e.g.*, Roundup<sup>®</sup>, Accord<sup>®</sup>) are commonly used as the PRIMARY or SOLE means for weed control.

- A. Agricultural fields (Please specify crops): 50.00%
- B. Conifer plantations 27.08%
- C. Tree nurseries 10.42%
- D. Orchards/vineyards (Please specify crops): 18.75%
- E. Poplar plantations 25.00%
- F. Riparian Areas 18.75%
- G. Upland forests 12.50%
- H. Roadsides/rights of way 29.17%
- I. Other (please specify) 8.33%

Comments:

**3:** Native Ponderosa pine grows with cottonwood near Albany; Oregon ash also occurs in poplar plantations. Poplars can be a MAJOR weed of orchards. Roundup ready poplars could become a big issue for corn growers, for example

**9:** Spot treatment in hame gardens

**12:** glyphosate is widely used in agriculture industrial and forestry. In forestry it is commonly used for site prep and at lower rates is used for conifer release. Because it is broad spectrum it is less commonly used in nurseries. In roadside rights of way it is often tank mixed with other chemicals that have soil residual activity. In agriculture it is used to "sanitize" fence rows and as a site prep prior to tilling Glyphosate is not registered for use in poplar plantations ( fallow only). In Roundup formulation the label states "do not get in water, toxic to fish" actually it is the surfactant that is toxic to fish thus the Rodeo formulation is labled for aquatic plant control.

**14:** Not experienced enough to know.

**16:** Products are selected by determining type of weed to control and season of year. Rarely is a product "primary".

- 20:** Note: Roundup is currently in poplar plantations by some in a shielded or directed application during the growing season or (unshielded) just prior to the growing season and usually in a tank mix with pre-emergents.
- 24:** Roundup will seldom solve all weed problems alone. Generally other herbicides are also used.
- 25:** None in this area. Inappropriate since the spectrum for weed control is too limited and/or the damage on non-target species is too great.
- 26:** No knowledge
- 30:** Glyphosate is used in each of these settings. The type of crop and time of year will dictate whether glyphosate is the primary means of control.
- 31:** This is another loaded question. Herbicide choice is often a matter of grower preference. Where one grower may prefer Roundup, another may choose Liberty or something else. You'd be better off asking actual nursery managers and growers this question.
- 34:** While at times glyphosate may be used in all settings, other herbicide options exist that control weeds and do not increase costs.
- 44:** None. Usually it is included in tank mix used to target more than one weed species
- 46:** There is a difference between "primary" and "sole." I know of no setting where glyphosate is used as the sole source of weed control.
- 47:** Glyphosate is used in pretty much all of the above settings, but is hardly ever either the primary or only herbicide used. Because it is strongly non-selective, its use must be prescribed carefully to avoid damage to non-target plants.
- 48:** Canadian forests

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**Question 12**

Please indicate the major kinds of herbicides that are most often used TOGETHER WITH glyphosate-based herbicides (e.g., Roundup<sup>®</sup>, Accord<sup>®</sup>) for weed control (list them).

A. Agricultural fields:

**7:** simazine **16:** 2,4-d **18:** 2,4D **19:** phenoxy acids and other growth regulators, **20:** 2,4-D compounds for the broadleaves **21:** None as tank mixes. **24:** Soil-active persistent; phenoxy; almost anything else. **28:** 2-4 D **30:** 2,4-D, Dicamba **44:** Oust, sinbar, diuron, goal **47:** treflan, atrazine, simazine, lasso

B. Conifer plantations:

**6:** Garlon, Oust, Escort, Atrazine, 2,4-D **8:** Oust; Escort **10:** Arsenal, Oust **11:** arsenal, oust **12:** Herbicides containing tryclopyr ie Garlon **15:** crossbow **20:** In Canada usually no mixes **23:** Hexazinone and 2,4-D, Garlon **34:** for release: with 2,4-D, pronone(velpar) **37:** Arsenal, Oust, Escort **41:** Arsenal,Oust **42:** 2,4-D **47:** 2,4 D, arsenal, velpar, Oust, Garlon

## C. Tree nurseries:

**6:** Garlon, Oust, Escort, Atrazine, 2,4-D **15:** goal **20:** Probably with a pre-emergent in dormant season **21:** None **47:** soil sterilants (Vapam, methyl bromide), Poast, Goal, Surflan

## D. Orchards/vineyards: 0

## E. Poplar plantations:

**5:** Goal, Oust, Karmac **6:** Garlon, Oust, Escort, Atrazine, 2,4-D **8:** Oust **12:** not registered for use in poplar plantations (fallow use only) **20:** Oust, Sinbar & Diuron/Karmex, 2,4-D (shielded) **23:** 2,4-D and Transline or Stinger **28:** Goal **34:** 2,4-D, milestone(preemergent), **42:** 2,4-D **43:** 2,4-D products **44:** oust, sinbar, diuron, goal **46:** 2,4-D **47:** treflan, sceptor, fusilade, 2,4 D, transline **48:** Surflan, Septer, Goal, Treflan, Karmex, Sinbar

## F. Riparian Areas:

**6:** Garlon, Oust, Escort, Atrazine, 2,4-D **12:** Roundup is toxic to fish( the surfactant is ) . Rodeo is not **47:** believe herbicide use in these areas to be very limited

## G. Upland forests:

**6:** Garlon, Oust, Escort, Atrazine, 2,4-D **10:** Arsenal, Oust **12:** Mixed with Garlon for woody species control, mixed with oust for grass/weed control, Used to also be used with Atrazine but the label had been canceled because of groundwater concerns ( now it is mixed with Velpar instead) **20:** Usually no mixes in Canada **40:** goal, atrazine, Arsenal **47:** same as conifer plantations, but use is less extensive

## H. Roadsides/rights of way:

**6:** Garlon, Oust, Escort, Atrazine, 2,4-D **8:** Garlon 4 **16:** 2,4-d **34:** transline **47:** Paraquat, 2,4, D, arsenal, garlon

## I. Other (please specify):

**28:** Rangeland- Tordon

## Comments:

**3:** Endless combinations! Poplars tend to be LESS TOLERANT than other trees. Wide range of herbicides kill it, and roundup is NOT the best

**16:** May use fertilizer to improve effectiveness of round-up

**17:** In the ag field applications that I see glyphosate used it is mainly used alone.

**20:** Big differences between US and Canada. Tank mixes listed above are in the US only.

**31:** I can't really answer this question as the herbicide mixtures used are often a matter of grower preference or necessity (if there's resistance).

**33:** I almost always use glyphosate by itself.

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**Question 13**

Please indicate the major means employed to control wild poplars in areas with which you are familiar. Choose all that apply.

- A. Glyphosate-based herbicide (*e.g.*, Roundup(r) , Rodeo(r)) 41.67%
- B. Other herbicides 31.25%
- C. Repeated tilling/cultivation (*e.g.*, between rows) 50.00%
- D. Combination of herbicides and cultivation 31.25%
- E. No control attempted 27.08%
- F. Other (please describe) 29.17%
- G. Don't know 18.75%

**1:** cut it down, remove root system **2:** burning: esp. in clearcuts **21:** mowing **31:** "ground-pruning" **43:** hand pulling / mowing **46:** mechanical **47:** hand weeding  
G. Don't know 9

Comments:

- 1:** No control in fence rows, drainage ways;
  - 2:** No control on govt. land
  - 3:** Glyphosate-based not preferred 2,4 D, Garlan mix; Crossbow (2,4,D and triclopyr. Herbicides and cultivatoin for row crops like cauliflower Probably no control attempted in western Washington; perhaps a fall application of Garlan
  - 7:** preemergents
  - 14:** Cultivation is commonly practiced in SE and Midwest USA.
  - 17:** I have no experience with poplar control efforts.
  - 23:** In our area, there is very little if any control of wild poplars in our conifer or hardwood plantations.
  - 25:** Not a problem.
  - 28:** Wild poplars do not occur in this area unless there is a source of water such as along a creek or spring. Native cottonwoods will sprout where there is adequate water along ditches and at the bottom of row crop fields- these are controlled with 2-4 D when needed.
  - 30:** This is our second attempt at growing poplars. We have not used chemical control but would not rule out the possibility. As for other local fields, I'd assume the primary means of control is also mechanical.
  - 34:** This is not a significant problem for us on the eastside. We do not change our management practices to control wild poplars. Wild poplars/aspens have not been a problem in the five years of our poplar assessment in MN.
  - 41:** popular not typically a competitive problem with conifer est. in upland situations- Alder and maple are more common
  - 46:** As you know, on the eastside, we don't have a problem with wild poplars establishing themselves on the farm. Occasionally, a lombardy escape will land in a fallow area on the farm-we won't notice it until it is chainsaw size. After cutting it down, we'll go in and lop off any sprouts that arise.
-

**Question 14**

Glyphosate-tolerant wild poplars would be significantly more difficult to control than glyphosate-susceptible wild poplars in areas with which you are familiar.

- A. Strongly Agree 8.33%
- B. Agree 27.08%
- C. Unsure/No Response 27.08%
- D. Disagree 27.08%
- E. Strongly disagree 10.42%

Comments:

**2:** Depends on herbicide regime: mixture of imazapyr/glyphosate is very common; need resistance testing for poplars; most west-side forest release and site prep operations use Oust for state/private land; poplars are one of the worst problems for forestry on federal land in western washington; no control allowed in riparian areas

**8:** Would use Garlon 4 instead.

**12:** Use a triclopyr or 24-D based chemicals

**14:** I'm unsure mainly because I'm sorely out-of-date on the alternatives to glyphosate. If none (or "no good ones" or no economically feasible ones) exist, then I believe a case could be made for a control problem. However, if glyphosate-tolerant plants are still sensitive to another effective, affordable (and legal!) brush control, then I see no major problem. One of the big problems of course is that Populus is so closely associated with fresh-water supply sources.

**16:** There are other products that will kill poplars, 2,4-D, Tordon, etc.

**20:** Lean towards disagree, depending on the setting I am in. In a forest environment there may be some limitations, certainly in Canada. Where cottonwood seeds in very heavily, there are usually other weed species that also pose a problem. For conifer plantations there are several other non-glyph. options, especially in the US. For poplar plantations there would be no problem.

**24:** Something like triclopyr would be more likely used, anyway.

**30:** Other compounds such as dicamba + 2,4-D, 2,4-D ester or triclopyr should give good to excellent control.

**31:** Depending on the setting and the control strategies used (*e.g.*, in agricultural fields), there is the potential for a problem. However, if the things are getting harvested before they flower (or if they don't flower), there won't be a problem.

**34:** Cultivation occurs up until canopy closure and from then on wild poplars have a very low probability of becoming established.

**37:** Especially in WA

**41:** Other herbicides are substitutes for control

**46:** As mentioned, on the eastside, we rarely see any wild escapes, and when we do, we use mechanical means to remove them.

**47:** Glyphosate is not the principal means now for controlling poplars in areas where they are unwanted. Cultivation or hand pulling are probably the most common. There is one notable exception. Some commercial poplar growers utilize glyphosate to kill

sprouts arising from the stumps of mature trees that were harvested. The new tree row is offset to run between the old tree rows, and the sprouts arising from the stumps are sprayed out with glyphosate. So, in this scenario, a different herbicide(s) would be needed. Fortunately, there are alternatives, such as 2,4 D, garlon, dicamba. More care would be needed to use these alternatives, but the problem is manageable.

**48:** Notised increased susceptibility to Garlon with r-r poplars

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**Question 15**

If glyphosate-tolerant poplars were to become common, the reduced usefulness of glyphosate for weed control would be a significant ECONOMIC issue in some areas (please indicate where).

- A. Strongly Agree 2.08%
- B. Agree 10.42%
- C. Unsure/No Response 39.58%
- D. Disagree 35.42%
- E. Strongly disagree 12.50%

Comments:

**3:** Poplars aren't a noxious weed; no potential to act as a weed in this climate: poplar is site-limited in Oregon

**4:** Such GT wild poplars would only be of concern in hybrid poplar plantations- but their growth rate would be inferior to the hbybrids and they would be outshaded

**8:** Garlon 4 is a more expensive chemical than Accord, but I wouldn't consider it significant.

**9:** I don't consider poplars a weed. So I don't need to control them very much.

**12:** Use of glyphosate resistant poplars in fiber farming situations like the lower Columbia and mid-Columbia eastside will allow more use of glyphosate for broad spectrum weed control between rows. Control of unwanted poplars can be accomplished using triclopyr or 24-D

**14:** Again, I'm not up to speed on alternatives and costs, but poplar is certainly only a small component of the spectrum of plants controllable via glyphosate application. And, again, the other side of the issue is cost of alternative control measures - there now exist other compounds that will kill unwanted poplars.

**16:** See No. 15. Other products are probably cheaper.

**17:** not familiar with poplar control programs but I would think if glyphosate is the primary means of controlling poplars now, then introducing a glyphosate resistant variety would have significant impact on control options.

**18:** not sure what you mean here. Is the glyphosate being used to control poplars or just a byproduct of its use on non-poplar weeds? If poplar is the weed, then I would agree. If not then why is glyphosates usefulness reduced. I'm missing something.

**20:** With the possible exception of conifer plantations in Canada, where no other herbicide options exist.

**21:** In this area the control of populars is not a major issue at this time.

- 22:** Would become issue for control of poplars, not other species
- 24:** Only poplars should be affected, and other herbicides could be used. No other weeds should be influenced.
- 27:** I don't know if there are alternative herbicides for poplar management, and if so, their costs.
- 28:** Water grass is a major weed problem in irrigated fields. Broadleaf herbicides won't work but roundup could still be used. As long as they are not 2-4 D intolerant then we can still control poplars.
- 30:** Possible concern might exist for roadside control, and controlling volunteer poplars in newly established plantations. It may cost more to control the tolerant clones in these settings.
- 31:** Again, this is very dependent on the setting/situation.
- 34:** The only area of concern is post harvest control of stump and root sprouting from RT poplars. Glyphosate appears to be the lowest cost alternative for controlling sprouting after harvest. Other chemicals could be used, but at a slightly higher cost to maintain effective control.
- 37:** The timing of seed production of poplar is the key. Seed is typically released late in the spring. Often as late as late May and early June. Many of the herbicides that effectively control seed germination in plantations begin to lose their effectiveness around this same timing (Oust, hexazinone). Thus, seed lands in an area somewhat free of competing veg. and can quickly establish. Also, harvesting methods, typically those like skidder logging, which bury broken fragments of branches of cottonwood often increase the establishment of multitude of sprouting trees. These typically are quite resistant to residual herbicides such as Oust, and hexazinone. Thus, fall applications of Accord is a major tool used to control invasions of problem cottonwoods. Again, this is a problem which occurs primarily in parts of WA state and not so much in OR.
- 38:** Glyphosate will still be good weed control in most areas. Currently poplar plantations are not being planted in my area (Spokane) But there are several people who are interested. And I don't see an economic issue of glyphosate tolerant poplars coming up due to most of the areas that are targeted for plantations that I know of are surrounded by fields or forests.
- 41:** low land margins of ag fields
- 47:** Glyphosate is not the primary means to kill poplar in areas where it is unwanted.

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**Question 16**

If glyphosate-tolerant wild poplars were to become common, the reduced usefulness of glyphosate for their control would be a significant ENVIRONMENTAL issue in some areas (please indicate where).

- A. Strongly Agree 2.08%
- B. Agree 31.25%
- C. Unsure/No Response 29.17%
- D. Disagree 29.17%
- E. Strongly disagree 8.33%

## Comments:

- 3:** Because of invasion of native populations
- 4:** To my knowledge, glyphosate is not used in environmentally sensitive areas
- 8:** Garlon 4 is not significantly more detrimental to the environment than glyphosate.
- 11:** Loss of round-up as a means of controlling poplars in itself would hardly be an environmental crisis. It could be argued that a loss of roundup effectiveness would lead to the use of other, more harmful chemical or mechanical weed control methods. Nonetheless, I am concerned about potential escape of this or other pesticide resistance.
- 12:** see last comment
- 14:** Once more, I don't have enough knowledge of what's now used and/or licensed for this application. Obviously, a compound(s) that is long-lasting in aquatic environments would present substantial problems.
- 15:** riparian zones?
- 18:** Not in semiarid systems that I am aware of.
- 20:** The outcrossing might be perceived as an issue, especially where natural populations of cottonwood are located. It may be that glyphosate resistance gene(s) will be diluted so enormously that it will make no difference. It would be interesting to test how well "natural" resistant trees compete with "truly wild and unpolluted" trees. I always point out the example of the Lombardy poplar, which has been planted widely throughout the PNW. Certainly there are hybrids formed with trichocarpa, but I have never seen these hybrids dominate the cottonwoods of the same age in seeded-in blocks. The amount of recognizable hybrids is very low and they are usually average in height and vigour compared to the trichos.
- 22:** Might require use of more resistant or dangerous chemicals for control.
- 24:** See above.
- 27:** The use of glyphosate and possible alternatives are hazardous to the environment. If glyphosate-tolerant trees became established in the wild, more environmentally-hazardous herbicides might be "designed" to control them -- should control be required.
- 30:** As long as there are other means of effective control, I can't see why there would be an environmental concern.
- 31:** There is that potential, perhaps in areas where native habitat is being restored.
- 34:** While we do not see control of wild RT poplars as a problem, glyphosate is one of the most environmentally benign herbicides and alternative herbicides could pose a larger environmental issue.
- 37:** Again in WA
- 38:** In my areas noxious weed control is a problem, and we generally don't use glyphosate since it kills all species, and we are trying to maintain as many species as possible except for the noxious weeds. So we favor leaving the grasses. Generally we are using Tordon or 2-4D.
- 47:** This is more problematic. Because glyphosate has no soil activity and breaks down rapidly, it is considered to be very benign from an environmental point of view. Other substitutes for glyphosate run the range of persistence in the environment, and hence it is possible that a glyphosate substitute could have a more negative effect on the environment. One might also argue that if cultivation increased because of glyphosate resistance, there might be an increased risk of soil erosion from the wind.

**Question 17**

Insect damage is common and extensive (*i.e.*, detrimental to growth or survival) in poplar PLANTATIONS.

- A. Strongly Agree 12.50%
- B. Agree 35.42%
- C. Unsure/No Response 35.42%
- D. Disagree 14.58%
- E. Strongly disagree 2.08%

Comments:

- 3:** Insect damage pretty common, but not extensive: localized problem. Disease is a much bigger problem
- 4:** especially in the more continental climates of the world
- 8:** We have not experienced significant insect damage in Willamette Valley plantations.
- 9:** so I am told
- 11:** I am aware of extensive damage to plantations in the area, but am not sure how frequent, and have not seen such damage locally.
- 12:** Refer to USDA Ag Handbook 654 (1990) Silvics of North American Hardwoods for insects
- 14:** The problem can be severe depending on intensity of the infestation and varies from place-to-place and year-to-year
- 16:** Our test plantation has had to be sprayed annually since it was planted 4 years ago.
- 17:** have no ideano
- 20:** In the coastal environment of BC and Oregon/Washington there are occasional serious insect outbreaks, but they are usually temporary as natural predators and control mechanisms build up and control damaging insects. I have witnessed this with the sawfly infestations in SW BC and western Washington. Populations build up, do considerable damage in terms of defoliation, but are usually controlled through natural means within a year or so. We have never considered use of insecticides as necessary. The story may well be very different in Eastern Wa/Or and possibly other regions. For saw timber and veneer, borer insects will be a problem, especially in the drier regions and now that all companies are switching over to saw log and veneer log production!
- 21:** Some alternate host problems do exist with some tree fruits.
- 23:** In some locations and situations.
- 27:** Poplars have a high economic injury threshold once they become established -- after first 3-4 yrs.
- 28:** Borers affect weak or stressed trees- healthy trees will grow past the borers as they did this spring in our area. Leaf beetles can be controlled when the population is high enough with aerial spraying.
- 30:** We haven't observed any significant damage from insects. Deer and other browsing animals seem to cause the most extensive damage.
- 39:** I do not have first hand knowledge of the impact of insects in poplar plantations, but I have been told by others that it can significant. Based upon my general knowledge of

insect biology/population dynamics, I would anticipate that insect problems will become more frequent and severe as intensive poplar culture becomes more common.

**40:** common and extensive, but how much of npp is consumed? May or may not be important

**47:** Insect infestation is common, but damage is not. This is because most growers aggressively treat insect infestations with a variety of insecticides, either by air or via irrigation systems. Untreated poplar plantations would have extensive damage and much reduced growth from insects.

**48:** Mostly eastside

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### **Question 18**

Insect damage is common and extensive on WILD poplars.

- A. Strongly Agree 4.17%
- B. Agree 27.08%
- C. Unsure/No Response 39.58%
- D. Disagree 27.08%
- E. Strongly disagree 2.08%

Comments:

**3:** Not apparent in native stands

**9:** I haven't noticed insect damage on *Populus trichocarpa* in Western Oregon

**12:** Most insects do not differentiate between wild and planted trees. They are however host specific and population specific. ie epidemic outbreaks are more likely where there are large amounts of host plants in close proximity as in plantations.

**14:** One needs to look more closely, mainly because of the generally larger size of trees, but damage is often extensive. Disease is same.

**16:** Insect damage on wild poplars is rarely detrimental to the trees long term health. The concern in plantations is modifying tree shape, growth or wood quality.

**20:** On the coast not so much, although we have seen periodic infestations (as an aside, the hybrid poplar plantations fared really well during these outbreaks). In the dry interior there is much more damage potential.so.

**23:** In some situations and locations. Not every year or in all locations or age classes.

**27:** Poplars tolerate LOTS of damage once established.

**30:** Haven't taken the time to thoroughly assess any damage on wild poplars. I'd assume that aphids might be a concern.

**31:** Somewhat cyclical in the upper Midwest.

**34:** I've never examined wild poplars for damage.

**36:** A different complex occurs in wild vs plantation poplars. Damage in wild poplars is episodic. More chronic in plantations. More common and more extensive in plantations. In a few instances,

**37:** Does seem so from my casual observations

**39:** This is a difficult question to answer. There are certainly many insects associated with wild poplars, some native and some introduced. At times the populations of these insects may reach levels at which they significantly impact the survival and growth of poplars in some areas. Whether this is considered "common and extensive" damage is a matter of judgement. The answer depends upon what those wild poplars are managed for and the extent to which the insect activity interferes with those management objectives.

**40:** ditto

**47:** The degree of damage obviously varies by year, but certainly wild poplars maintain higher insect populations and sustain more damage than ever would be tolerated in intensively managed poplar plantations.

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**Question 19**

Please indicate the major means employed to control defoliating insects of poplars. Choose all that apply.

- A. Spraying of *Bacillus thuringiensis* (Bt) based insecticide (e.g., M-trak<sup>®</sup>, Dipel<sup>®</sup>, Condor<sup>®</sup>, Javelin<sup>®</sup>, or MVPII<sup>®</sup>) 18.75%
- B. Spraying of insecticides other than Bt 45.83%
- C. Integrated Pest Management, including introduction of natural predators 18.75%
- D. No insect control attempted 20.83%
- E. Don't know 35.42%

Comments:

**3:** BT for tent caterpillars: almost the preferred option

**12:** refer to PNW 356 "Hybrid Poplar Plantations in the Pacific Northwest"

**16:** Use of insecticides is done under IPM guidelines. Insect population monitoring is mandatory.

**20:** On the coast "we get away with no insect control". Integrated pest management would be best, but this approach is still in its infancy. I have a concern that in areas with a strong agricultural crop base (such as in the Columbia Basin), extensive use of insecticides by all crop growers, including poplar farmers, increases insect problems and strengthens dependency on these compounds. The natural system has probably been set back severely and only time and careful planning of an IPM approach can reverse this. In Europe there is little if any foliar spraying to control insects on plantation poplar. In Italy there are stem applications of insecticides to control borers.

**27:** Incidental spraying of poplars w/Bt-based products for control of FTC or GM, but conventional insecticides are used in plantations because costs of Bt are too high.

**28:** We have had very little problems with insects so far. The leaf beetles were sprayed when several generations had pupated. In the fall the migratory birds such as chickadees, juncos and cedar waxwings have taken care of the beetles.

**30:** I can't speak on behalf of other growers, but we have not used any chemical means to control insects. I'd assume Orthene, Diazinon, Sevin would provide acceptable control on most insect pests.

**31:** I remember them using a snowplow one year in Minnesota because the forest tent caterpillar outbreak was so bad that they were covering roads making them slicker than ice.

**39:** My answer to this question is an educated guess.

**47:** As pointed out earlier, insecticides are also applied via irrigation systems.

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**Question 20**

Do you believe that genes for resistance to defoliating insects in wild poplars would make them significantly more difficult to control where they are undesirable? (In answering this question, please assume a 10 to 20% enhancement in growth for insect-resistant wild poplars compared to non-resistant trees).

- A. Very likely 0
- B. Likely 8.33%
- C. Unsure/No Response 22.92%
- D. Unlikely 47.92%
- E. Very unlikely 20.83%

Comments:

**2:** BT doesn't provide very impressive resistance to insects

**3:** Limited availability of establishment sites in Oregon; Moisture, soil type, competition, girdling by mice; Probably different for western Washington

**11:** Do not see them as a weed problem here.

**12:** insect resistance and herbicide resistance are not likely to be controlled by the same genes

**14:** Hey, who now controls unwanted poplars by siccing insects onto them anyway?!

**15:** don't know the chemistry that would make this possible or the reaction of the insects to becoming immune to the product and damage the trees anyway

**16:** question is not clear to me.

**18:** Depends on the linkages

**20:** Trick question? Even if poplars in the wild have Bt genes for instance, they are still susceptible to chemical herbicides and/or the powersaw!

**27:** Poplars are relatively short-lived trees. Insects rarely cause tree mortality once established.

**30:** I know that insects resistant to Bt would be difficult to control with Bt, but the efficacy of other compounds shouldn't be effected.

**45:** Does "them" refer to controlling the genes, the insects, or the trees? I am assuming you mean the trees--but this evades the question about whether insect-resistant genes might make the wild poplars more competitive, and therefore more likely to become invasive. In other words, requiring control measures where they might not otherwise have been needed. Not enough is known about the effects of insects on poplars to answer this latter question.

**47:** The assumed growth advantage would not make such poplars more difficult to control.

**Question 21**

The use of varieties of poplar trees that are unable to produce viable seeds or pollen should be a requirement for the use of herbicide or insect resistant poplars produced via genetic engineering.

- A. Strongly Agree 16.67%
- B. Agree 39.58%
- C. Unsure/No Response 27.08%
- D. Disagree 14.58%
- E. Strongly disagree 2.08%

Comments:

**9:** Humans must not upset the complex interrelationships in the natural world that keep it functioning. Insects that eat cottonwood are part of the same web we are.

**12:** probably wise but not a necessity and not enforceable anyway

**14:** It would be useful to have sterile genetically-transformed trees, but I'm not sure it is a requirement. If it is, I can understand the case for herbicide resistance-linked sterility more clearly than I can the insect resistance-linked case.

**20:** This is one for risk assessment. In the absence of hard data to carry out this risk assessment, the safest route is not to do anything or to have sterility. One day we may regret the cost of achieving this, were it not so attractive from a purely scientific viewpoint. It would be nice to have the ability to sexually re-produce engineered traits, since various genotypes may be recalcitrant to genetic modification, e.g modify trichocarpa, cross with deltoides and voila, a resistant F1, with possibilities for advanced generation work! From an environmental point of view it would be nice to have sterility. What we need is a mechanism to allow fertility on command!

**22:** Even sterile trees will still reproduce and spread vegetatively.

**27:** Optimally, poplars should now produce pollen at all since they are wind pollinated and "toxic" pollen increases risk to nontargets.

**31:** I agree for a couple of reasons: 1) the potential loss of effectiveness of Bt resistance genes should they become common in the environment. You'll want to keep that buffer of susceptible plants or you'll lose your gene that much sooner. 2) Herbicide resistance will create a problem in, e.g., riparian areas, where glyphosate is one of the few herbicides available for use in, or that close to, waterways. 3) It'll be a lot easier to get the trees deregulated if there is no fear of "contaminating" native trees with the transgenes.

**32:** If these poplars are grown in regions without native or wild poplars, then sterility would serve no purpose. But if wild poplars are present, then movement of genes from plantations to wild populations could be a real problem (both for control of the poplars and for maintenance of any "genetic purity" of native species).

**34:** While we can make a strong argument for non-sterile poplars on the eastside, regulatory aspects may require sterility. Sterility would also make GM plant materials more acceptable to our PR and upper management.

**35:** It would be preferable, if reduced sexual activity did not negatively affect growth rates....

**43:** At least for use east of the Cascade Range.

**46:** It depends where you plan to grow them. As members of the Salicaceae, these trees exhibit cladopsis. Even if you had sterile trees, you still run the risk of transgene escape via a branch breaking off during the rainy season and establishing itself elsewhere. This could happen on either side of the Cascades. Serious consideration of this statement is a probability game. I think that on the eastside, the probability of escape is low even without sterility--but the possibility exists.

**47:** I believe this should be determined based on an analysis of the environment into which such trees might be released. For example, east of the Cascades the area suitable for establishment of poplar seedlings is very limited. In those areas where poplar can be established, control is very easily accomplished by means other than those that depend on glyphosate. The one possible exception is a riparian zone. These areas are also limited on the eastside, and wild poplar seedling establishment is not controlled. Indeed, one might argue that wild poplar ought to be encouraged in riparian zones to help protect against soil erosion. Establishment of poplar seedlings in a riparian zone occurs, but with great difficulty, owing to the rapid drying-out of suitable seedbeds and extreme competition from herbaceous weeds. It is difficult to imagine that poplars containing a glyphosate resistance gene would have much competitive advantage over wild seedlings. Furthermore, because poplar seedling control is not practiced in riparian zones, the issue of loss of glyphosate as a tool does not apply. Having said all this, I am well aware that this is an issue that will be controlled more by politics than by science. Thus, it would be prudent to continue research into engineered sterility, all the while doing environmental risk assessment studies to determine the potential effects of glyphosate resistance poplars released into the environment.

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**Question 22**

The use of sexually fertile herbicide or insect resistant poplars produced via genetic engineering is likely to cause only minor, manageable impacts on agriculture, forestry, and the environment.

- A. Strongly Agree 8.33%
- B. Agree 35.42%
- C. Unsure/No Response 37.50%
- D. Disagree 18.75%
- E. Strongly disagree 0

Comments:

**9:** wishful thinking. especially about insect-resistant

**11:** I don't think we know. Risks may be low, but stakes could be high. Best to stick with steriles for now.

**12:** key word is manageable

- 20:** I have to agree, although still somewhat on the fence. As we proceed with the increasing domestication of poplar (as outlined by Toby at the TGERC/PMGC meeting) sterility becomes less of an issue. So over time, with progress, I agree!
- 24:** The major impact would be in developing insects that are resistant to Bt, so it could not be used for other purposes.
- 27:** Bt toxins, although narrow spectrum compared to conventional insecticides, will impact other herbivorous insects beyond the targets. Although most people don't object to a reduction in insect numbers, LOTS of beautiful butterflies and moths feed on Populus trees. In addition, impacts on predators, parasites, etc. should be looked at more thoroughly.
- 31:** There is the potential for problems. That's what "white papers" are for. This issue needs to be addressed with further research.
- 32:** I am unsure because these effects seem difficult to predict. What cause me the greatest concern about release of genetically engineered organisms is unanticipated effects, and these, of course, are not predicted. An example is pollen from BT-engineered corn having a pesticidal effect on downwind wild lepidopteran populations (at least, so I read in the paper!).
- 35:** Effects are somewhat situationally dependent, thus I'm unsure on this point.
- 37:** Removing one of our best tools in terms of efficacy and environmental safety seems to be short sighted. Other herbicides can take its place but for how long. When will future engineering make the trees resistant to these as well.
- 40:** What is the "environment?" there could be a shift in gene frequencies in poplar.
- 47:** I'm taking a calculated step here that such engineered poplars will not become the next version of kudzu. Such poplars will have some advantages over non-engineered trees. However, I do believe these advantages are not large enough to create a tree that suddenly will become uncontrollably weedy, or even result in significant increases in weed control costs. Again, having said the above, this is something that will need to be studied and a risk assessment completed.

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**Question 23**

Varieties of poplar trees that have a greatly reduced ability to produce viable seeds or pollen would present significantly less environmental or economic concern with respect to the spread of engineered genes.

- A. Strongly Agree 25.00%
- B. Agree 39.58%
- C. Unsure/No Response 22.92%
- D. Disagree 10.42%
- E. Strongly disagree 2.08%

Comments:

- 3:** I also appreciate the opposite view: we could be producing 'exotic' organisms with genetic engineering. Genetic mixing is the strongest negative argument

- 11:** Reducing the numbers would likely reduce the odds of escape, as in any crap shoot.
- 12:** speculative
- 14:** This is especially true at present when we have very little information on spread or impacts of sexual recombinations of engineered genes in wild populations. Sterility/reduced fertility would allay the fears of many persons and agencies.
- 20:** In light of question 22, I have to be "unsure". I would have a hard time significantly reducing risk when I find the risk already insignificant to begin with!
- 21:** The main spread in this area has been via suckers which are derived from the evasive root system.
- 22:** Vegetative reproduction would still occur and become significant means of dispersal.
- 28:** We are harvesting poplar trees in eastern Oregon at up to 10 years of age so the tree will probably not be producing much seed or pollen yet?
- 45:** The magnitude of the concern depends on the characters imparted by the inserted gene.
- 46:** I agree with this statement for west of the Cascades and I disagree with this statement for east of the Cascades.
- 47:** The percentage of viable wild poplar seeds that actually make it through the environmental gauntlet to actually become sexually mature poplar trees is very low. Thus, if engineered poplars have significantly reduced fertility, this would increase the odds again. This is something that needs additional research.

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**Question 24**

The vegetative spread of genetically engineered poplars via root sprouts or rooting of branches is likely to cause only minor, manageable impacts on agriculture, forestry, and the environment.

- A. Strongly Agree 18.75%
- B. Agree 39.58%
- C. Unsure/No Response 31.25%
- D. Disagree 8.33%
- E. Strongly disagree 2.08%

Comments:

- 11:** so long as they are sterile plants...
- 12:** Impacts are manageable because poplars are easily controlled by more than one herbicide
- 14:** Aspens are a possible exception.
- 27:** Branches breaking off over waterways should be avoided to restrict long-distance spread. Otherwise, vegetative repro. is not a big issue.
- 30:** I'd assume the spread of poplars via vegetative means is minimal.
- 32:** This depends on the dispersal ability of root material (*e.g.*, during flood events) and the competitive ability of genetically engineered trees.
- 36:** Depends on the level of management. Cannot answer without that context

**38:** This could be a problem to the plantation, if the tree escapes outside of the plantation and the escaped clone is spayed in has the potential to kill the tree in the plantation. The problem I see is mainly damage to the plantation.

**45:** Depends on the character of the inserted gene.

**46:** On the eastside, I agree with this statement....but on the westside, I can envision scenarios, (that are improbable) that could have environmental effects that are not minor, particularly with the Bt gene. If a field containing transformed trees were planted by a nearby stream or river, there would be no way to determine the extent to which the gene had escaped.

**47:** This is not a problem at all on the eastside. On the westside it could be a minor problem if some engineered trees were flooded and uprooted.

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**Question 25**

Economic and environmental benefits from the use of sexually fertile herbicide- or insect-resistant poplars produced via genetic engineering outweigh their potential negative impacts on agriculture, forestry, and the environment.

- A. Strongly Agree 4.17%
- B. Agree 39.58%
- C. Unsure/No Response 39.58%
- D. Disagree 8.33%
- E. Strongly disagree 8.33%

Comments:

**9:** wishful thinking. But once they are out in the wild it is too late to stop. There isn't even a half life. It could be a curse.

**14:** I need to see more research before I can strongly agree!

**15:** need better definition of sexually fertile herbicide

**20:** Somewhat agree

**34:** The jury is still out on the economics of RT poplar, but insect resistant poplars should be favorable economically and result in much lower pesticide use.

**35:** I am a borderline case on this question, as I somewhat agree but would like to see more data/research in this area.

**36:** Insufficient data available to answer

**38:** At this time I agree but a lot depends on the exact genetically engineer tree and how it will or not adapt.

**40:** This isn't a question of science. It is a balance of competing personal values.

**41:** Poplar is a minor species in forest plantings now and I see little increase

**46:** My answer depends on whether the statement is referring to herbicide resistant or insect resistant trees. If an herbicide resistant escaped and established itself in the riparian areas, so what? No one is spraying herbicide in riparian areas, so the selection force that would favor that tree would be absent. However, there are beetles in riparian

areas. You could change the genetic structure of "natural" populations of trees and beetles in riparian areas.

**47:** If plantation growers had poplars transgenic for a Bt gene or genes, exogenous application of much more toxic and persistent insecticides could be eliminated entirely. This can have nothing but positive impacts. A similar argument can be made for glyphosate resistance, although the time frame is different. Weed control in poplar plantations on the eastside is only necessary for 2 years; on the westside it is 3 years. Once there is crown closure, weed control is no longer needed. So, under either scenario, herbicide use in poplar plantations is much lower than in annual crops; switching to glyphosate only should have a positive impact on the environment.

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**Question 26**

Glyphosate-based herbicides (e.g., Roundup<sup>®</sup>, Accord<sup>®</sup>) should NOT be used in poplar plantations because of their detrimental effects on the environment (please explain).

- A. Strongly Agree 2.08%
- B. Agree 2.08%
- C. Unsure/No Response 12.50%
- D. Disagree 45.83%
- E. Strongly disagree 37.50%

Comments:

**1:** It's a question of degree: shift of weed populations a concern

**9:** These herbicides are quickly biodegraded.

**11:** Glyphosate use in poplar plantations is not an environmental hazard.

**12:** as of now this is a non-registered use. It is not on the label because of potential damage to the poplars, not because of environmental concerns. Roundup is an environmentally benign chemical. Refer to OSU Extension Glyphosate Fact Sheet and MSDS. It is practically non-toxic to mammals its primary breakdown substance aminomethylphosphonic acid is also practically non-toxic, in test rats over 90% of ingested dose is passed through the system within 24 hours. there is no evidence of it causing birth defects, nerve damage, cancer or DNA damage. it is practically non-toxic to birds and honey bees. It is slightly toxic to fish because of its surfactant, it does not bioaccumulate, it is stable in water and stable in sunlight, it is degraded by aquatic organisms in 14-21 days and in soil within 47 days. it ties up readily in organic matter and thus is unlikely to move. It is one of the most tested herbicides in existence,

**15:** It is my understanding that glyphosate breaks down more quickly than some other products, yes?

**18:** It is the surfactant in Roundup that is most damaging to aquatic systems.

**21:** Use should be based on the need of the material to control a specific pest, not blanket applications.

**24:** What detrimental effects??

**26:** Glyphosate degrades quickly enough to limit its environmental impact

**27:** I am concerned about the use of herbicides on the environment. Obviously, use should be limited to weed management while trees are being established ONLY. Weed management should be silvicultural or mechanical to minimize use of herbicides. This will increase costs and reduce yields, but the magnitude of herbicide in these plantations is daunting and easily abused to increase profits.

**30:** Glyphosate is an extremely mild pesticide. When used according to labeled directions the impacts to the environment are minimal.

**34:** Glyphosate is environmentally benign.

**35:** They are relatively short-lived herbicides with limited research demonstrating long-term negative effects....

**38:** I would like to say agree, but chemicals are one of those necessary evils we live with to supply society needs on a limited landbas and that is low cost.

**43:** There are herbicides other than Glyphosate-based products, and mechanical methods available for controlling any "escapes" (if there are any) that germinate in an undesirable area.

**47:** I an unaware of another herbicide that combines such broad spectrum control with benign environmental effects. Weed control is absolutely required in poplar plantations, so not controlling weeds is not an option. Glyphosate is an excellent herbicide that is used in poplar plantations, but it is by no means the only herbicide used.

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**Question 27**

Unanticipated and significant ecological or resource management problems are likely to be caused by the use of genetically engineered poplars in plantations (please explain).

- A. Strongly Agree 0
- B. Agree 6.25%
- C. Unsure/No Response 45.83%
- D. Disagree 41.67%
- E. Strongly disagree 6.25%

Comments:

**7:** I do not think genetically engineered material pose a threat to the environment

**9:** as long as the plantation trees are sexually sterile.

**11:** Because it has been using sterile plants, I have been much more comfortable with the use of genetically engineered poplar trees than I have been with the use of some fertile ag crop species, many of which have wild neighbors. My view would change if we were using fertile varieties.

**12:** see CAST "council for Agricultural Science and Technology" <http://www.cast-science.org/>

**20:** Plantations are highly controlled systems we can manipulate like a farmer can manipulate his fields and the crops on them.

- 21:** This is like answering the question "Have you stopped beating your wife yet." I could think of unanticipated problems at the level of the horror stories (*i.e.*, the movies of the mutant animals following nuclear testing in the 50's) and strongly agree. Other unanticipated problems could be relatively insignificant.
- 22:** Escape and spread of trees via vegetative means is certain to occur.
- 27:** We should proceed cautiously until fertility is eliminated just to avoid escape of genes to the wild. The impact on nontargets could be significant over the long run.
- 30:** You'll cross that road if and when it comes.
- 32:** see comments, above. It is inherently difficult to quantify the risks of "unanticipated" problems.
- 34:** One can never be 100% sure about the biological and environmental interactions in these managed systems, but we have a strong enough background proceed with large field trials to get information required for non-regulated status.
- 35:** Again, some additional research is needed in order to dispell the notion of unanticipated consequences....many times unanticipated effects can arise.
- 36:** Depends entirely on what steps were taken to prevent them. Question cannot be answered out of context like this. Depending on the steps taken or not taken, any answer from "agree" to "disagree" would be reasonable
- 37:** my crystal ball is fuzzy
- 38:** There is always the potential.
- 41:** Who know- there has been enough bad press recently on unanticipated problems with corn and soybeans that the issue may be more political.
- 43:** We carefully and thoroughly test all new plant material in small quantities before large acreage plantings are established. If an unanticipated problem occurs in this testing phase, it is usually not considered significant.
- 45:** Such a general statement! We wrote a whole paper on this question, if I recall. Doesn't it depend on the gene, at least? And how the trees are used. And where they are planted...etc.
- 46:** It depends on the gene.
- 47:** My training and experience tell me that such significant problems are very unlikely, but without more time working with such GMO's, we don't yet have enough experience to say one way or the other. Of course we can't prove the negative, that there will never be unanticipated and significant ecological or resource management problems. But, we should take the time to study this potential problem to make sure we have done a thorough analysis.
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**Question 28**

Scientific concerns aside, sexually fertile plantations of genetically engineered poplars are nonetheless likely to cause significant POLITICAL controversy that will impede their commercial use (please explain).

- A. Strongly Agree 18.75%
- B. Agree 47.92%
- C. Unsure/No Response 20.83%
- D. Disagree 12.50%
- E. Strongly disagree 0

Comments:

- 1:** Can't differentiate social from scientific issues; This is part of the technological treadmill, driving people from the land;
- 2:** Depends in large part on education level of public
- 3:** Enough of an element exists that don't want any alteration of the gene pool
- 7:** Possibly, people are ignorant about genetic engineering and fear the unknown
- 8:** There will be a media-driven assault against the use of transgenics due to public misperceptions.
- 9:** We are afraid of what we don't know. Every technology has its down side. The down side of spreading these genes in the wild populations could be great. You may think that this is political, but it is a broader science. Ecology
- 10:** The words "genetically engineered" cause POLITICAL controversy.
- 14:** Fear of the unknown is a difficult emotion to overcome!
- 16:** The uniformed public is always easy to scare. If I say that it will affect your children, I don't need science to prove my point and the media is always looking to make the "evil" chemical companies responsible.
- 20:** Perception is reality in politics. This requires significant public education and promotion. What is needed is a "feel good" story looking at the positive impact of genetically engineered poplars on society rather than the positive impacts on the producer of the trees (the farmer or multinational) or the company that markets the Roundup or sells the technology. I do not have a good example to use, but it has to do with setting aside precious "old growth" as natural-historical monuments, which is made possible because of the use of the high yielding poplars.
- 21:** Politics for sure. Unfounded fears induced in the minds of unknowledgable individuals can be easily propagated to a level of hysteria. There have been a number of instances with plants and chemicals that fear and hysteria have overridden knowledge. Unfounded fears easily lead to "political" solutions to "problems".
- 22:** Will cause public opposition.
- 23:** There may be political controversy that could impede commercial use, however, I think government regulations help dispel many of the fears people have.
- 24:** I don't think the man on the street cares about poplars. He might be concerned about gen.-engin. lettuce or other foods.

**26:** Genetically engineered organisms have gotten a very bad reputation, mostly because of incorrect "information" and fears fanned by "activists". There are plenty much more serious environmental issues which require our attention. "

**30:** Even if the science indicates minimal environmental impacts may exist, political and environmental controversy will surely exist ---- regardless of the science. You can look at Europe as a prime example.

**31:** In response to growing concerns about GMOs, a third, purportedly impartial agency with also participate in reviews of GMOs and petitions for deregulation. It is conceivable that even with GM poplars, there may be labeling requirements (see the story about protesters "confiscating" cotton undergarments in the UK that were supposedly made with GM cotton fibers).

**34:** From a business standpoint, most companies will proceed cautiously in this area until benefits to the environment (eg. reduced pesticide use) and cost savings are documented.

**35:** Just look at recent uproar over Bt corn and monarch butterflies.....when some additional research is clearly needed in followup to preliminary studies conducted by Cornell Scientists....

**38:** I don't believe so since we don't eat it, people won't have as much concern. A lot will depend on how much press this gets and if the press is supportive or not.

**43:** I think it will be difficult to educate the general public that the potential for environmental harm is slim to none. The public will perceive that when (not if) Glyphosate resistant seeds escapes from the plantation and germinate in their yard, they will not be able to control them.

**45:** It seems to me that poplar culture is small enough, and so little known to most people, that it is unlike to cause much political noise. Most people aren't even aware that poplar trees are grown in plantations, or for what purpose.

**47:** In this area (Columbia Basin), genetically modified annual crops have been in use for several years with absolutely no controversy. A perennial crop may be different, but I think that the controversy would be minor in this area. The much more populous (and politically left-leaning) westside is another matter entirely. I cannot imagine sexually fertile transgenic trees ever being de-regulated. Indeed, even with engineered sterility transgenic trees will have a much tougher go on the westside.

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**Question 29**

Scientific concerns aside, the use of genetically engineered poplars raises significant ethical concerns that need to be addressed before commercial use is permitted (please explain).

- A. Strongly Agree 4.17%
- B. Agree 33.33%
- C. Unsure/No Response 27.08%
- D. Disagree 27.08%
- E. Strongly disagree 8.33%

Comments:

**1:** The tool can be used ethically, but the marketing and product activities may be unethical

**3:** A common value set is that you don't mess with mother nature; It's bad for MAN to apply control to nature; this isn't a scientific view, but a sociological one.

**9:** If our science were complete, we wouldn't need ethics. But it never will be. One ethic says, "If you don't know how to fix it, don't break it.

**11:** I am not sure what the ethical questions are. Maybe, what should be the level of scrutiny given to GMOs? Should the approval be given based on the lack of evidence that something has gone wrong (the Nuke power argument) or on evidence of their safety (sort of the testing of new drugs). This relates to public trust.

**14:** I think that as many concerns AS ARE REASONABLY POSSIBLE TO ADDRESS need to be looked at before a significant commercialization is undertaken.

**15:** I believe that we need to have good non-biased research based information through several years of trials with good documentation and stats before going public so that unknowns are discovered and information is of high quality.

**19:** All technologies have unanticipated technical effects. They also have moral or ethical considerations that are usually overlooked or neglected. The questions of who benefits, who is harmed, who gets to decide, and what the externalities are are frequently neglected in agricultural decision making. I assume they are also neglected in forestry. Ethics is operative whether one knows it or not. What we ought to do is often determined by what is possible and the technological imperative to act. The foundational moral concerns are unrecognized, unexamined, or both.

**21:** The term genetically engineered is very misleading. It can mean inserting a gene from anything into an existing plant, animal, organism using highly technical procedures. However, if I find a poplar in the wild that has resistance to a specific insect, but is too weak to be used for windbreaks or pulp, and I cross it using traditional breeding techniques where I transfer pollen from one to the other, haven't I "genetically engineered" the resultant offspring. I think that these are two totally different concepts, but in the minds of some of the environmental activists, there is no difference. It is difficult therefore to answer this question.

**23:** I think the debate needs to continue, but not at the expense of halting the development of this technology, at least in plant biology.

- 24:** Changing food supplies needs care and study. I cannot see how poplars fits into a problem.
- 26:** The only ethical question that comes to mind is so-called "genetic pollution" of native poplar populations.
- 30:** You don't need another layer of bureaucracy! But undoubtedly a forum will evolve to tackle these ethical concerns.
- 31:** My personal opinion is that any field of science should be subject to a form of checks and balances. This applies to medical research, biological control research, you name it. If those checks and balances aren't used, bad science could result and with it, a loss of respect for and trust in science.
- 34:** Like most choices we have in life, you have to weigh the benefits of using engineered plant materials with the valid risks. If GE poplars allow us to produce more wood in a shorter time period and reduce our inputs of pesticides, the benefits are obvious.
- 35:** Im not sure on this one, the issue of ethical concerns really hangs upon the findings from additional research....
- 37:** Ethical issues are of course raised. I'm not sure what "need to be addressed means". These are emotional issues and likely addressing the impacts are completely out of your hands.
- 38:** I don't see a problem is genetically engineered plants. As long as it remains in plants.
- 41:** I think we need open discussions prior to planting. Also, there are enough other herbicides that I do not see growers begging for round up resistance. And the bt issue has not been a major limiting issue
- 42:** I'm not sure ethics has anything to do with genetically engineered plants.
- 45:** Well, see my paper... Mostly I am concerned about possible ecological impacts. Use good scientific judgement regarding what might cause a threat to ecological systems, including agricultural or forest ecosystems and don't just bend around scientific uncertainties to make false claims about safety. As far as the ethics of genetic manipulations in general sense (*i.e.*, 'playing God'), I don't have an ethical problem with that. But people have a habit of putting fame and money before what's "right".
- 46:** On the westside, I believe this to be the case. On the eastside, for Round-up Ready, I do not believe there are any ethical concerns that can not be readily diffused. As mentioned above, it is a probability game. There are countless other issues pertaining to agriculture that should raise greater ethical issues.
- 47:** There is nothing unethical about using technology to accomplish crop improvements in yield and culture. If the science supports such use, it would be unethical not to use it.

### **General Comments**

**4:** I think that manipulated sterility is a prudent early investment that will save many delays or setbacks later on. Conversely, the rush into wide-scale deployment of (fertile) GM clones could seriously backfire.

**9:** My answers were informed largely by Rick Meilan's talk to the SAF in Eugene. They were also informed by my personal experience and bias toward wilderness (from which we came). Thanks for doing this survey. I wish you well.

**14:** Sorry to be so conservative, but my knowledge base is somewhat dated!

**15:** Thanks for including me in your survey. sg

**17:** The grass seed industry is currently in a similar debate. And I am much more closely involved with that industry and its problems.

**21:** This apparently was originally sent to Clark Seavert, and he just forwarded it to me for response.

**31:** Steve, I would be interested in seeing the results of this survey. It would give everyone an idea of the general mood surrounding GM poplars. And, just a suggestion for any future surveys you may do that are related to this. Keep it completely objective and don't use a multiple choice format (although that's much easier to tabulate :). With this particular topic, only rarely will the answers be cut & dried, especially for someone in my position (regulator). The comments in this survey do not necessarily represent those of the Oregon Department of Agriculture.

**33:** I believe poplars are known to hybridize, not sure though. This would be my main concern, i.e. that engineered genes could be spread to native *Populus* spp. Also, while I use glyphosate as a preferred herbicide due to its relatively benign environmental effects, I use it in very small amounts and try to minimize use as much as possible. I am concerned about the development of glyphosate-resistant crop species, as it is likely to encourage use of larger amounts of the herbicide, *i.e.*, there will be less incentive to limit use. While I do not think small amounts of glyphosate use are an environmental concern, the use of large quantities is.

**35:** Good luck with your survey, I'd like to receive a copy of your results....also let me know how your research is proceeding and/or need for additional assistance w/riparian issues.....

**36:** In some instances, the wording of the questions made them difficult to answer. For example, some questions were compound in nature: "unanticipated and significant", etc. I would have responded to each of these differently, but they were sometimes embedded in one question. So I tried my best. Ken Raffa

**41:** 11

**45:** I skipped the glyphosate + management questions that I don't really have the expertise to answer.

**46:** I was frustrated with the style of this survey because my answers were situation-dependent.