

Assessing potential risks of transgene escape from fiber plantations

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ABSTRACT

Woody crops such as trees pose difficult obstacles for genetic risk analysis because of their long generation times, large size, and potential for long distance dispersal of pollen and seed. This necessitates reliance on established stands for retrospective studies of gene flow, and simulation models to assess the consequences of introducing new genes into the environment. We are using these approaches to assess potential risks of large-scale cultivation of transgenic hybrid poplar (*Populus spp.*). To predict gene flow from hybrid plantations, it is necessary to understand both the dynamics of gene movement within and between populations, and the ability of hybrid trees to produce fit progeny. We have found that up to 3.8% of progeny of wild females growing near hybrid poplar plantations were fathered by hybrid males. Also, hybrid seedlings established and grew at about the same rate as wild seedlings. Data from these field studies will be integrated in a spatial simulation model which is currently under development. The model will be used to identify the genetic and ecological parameters most important for predicting the spread of herbicide- and insect-resistance transgenes over space and time. Potential economic and agronomic significance of transgene spread will also be assessed.

INTRODUCTION

Forests cover more than one-third of the terrestrial landscape, and wood provides heat, shelter, and an array of other products for the majority of the world's inhabitants. Wood consumption has increased concomitantly with world population, and is projected to continue growing for the foreseeable future. For example, per capita consumption of fiber products increased 50% in the developed world and 300% in the developing world between 1970 and 1994 (FAO 1997). However, forests also harbor the majority of the earth's terrestrial species, and increasing pressure on native ecosystems is eroding that biodiversity (Wilson, 1992). Therefore, it is imperative to increase the productivity of managed forests and fiber plantations to meet burgeoning demand and alleviate pressure on wild forests (Dekker-Robertson & Libby, 1998). Genetic improvement of growing stocks is one strategy for enhancing productivity, and genetic engineering has the potential to make large contributions toward this goal.

However, despite its great promise, there are some risks associated with genetic engineering. These include the potential for increased invasiveness of the engineered organism, transfer of the transgene to wild relatives (leading to either increased

invasiveness or reduced fitness), direct effects of the new gene product on nontarget organisms, accelerated development of resistance to herbicides or insecticides by agricultural pests (e.g., Rogers & Parkes, 1995). Whether any of these possibilities pose threats significantly greater than are commonly assumed in conventional agronomic or forest management systems is, however, unclear (e.g., National Research Council, 1989).

Trees present some special complications for assessing risks of transgenics compared to herbaceous agricultural crops. For example, most trees have extended juvenile periods, long life spans, large stature, high fecundity, extensive gene flow, vegetative persistence or spread, and wild relatives that are spatially and genetically close to planted stock (James *et al.*, 1997). Therefore, risk assessments for trees must necessarily encompass long time frames and extensive areas, and spread may be highly dependent on rare events that favor colonization. We propose that simulation modeling must be a key tool for exploring the risk of transgene escape from tree plantations. Similar approaches have been taken to study invasions of exotic trees (Higgins & Richardson, 1998).

We are studying potential gene escape from transgenic hybrid poplar (*Populus spp.*, including aspens and cottonwoods) plantations. Poplar is a model organism for tree biotechnology, and is likely to be the first commercially released transgenic forest tree. Field trials are underway for clones transformed with herbicide resistance, insect-resistance, disease resistance, wood modification, and flowering control transgenes (Strauss *et al.*, 1998).

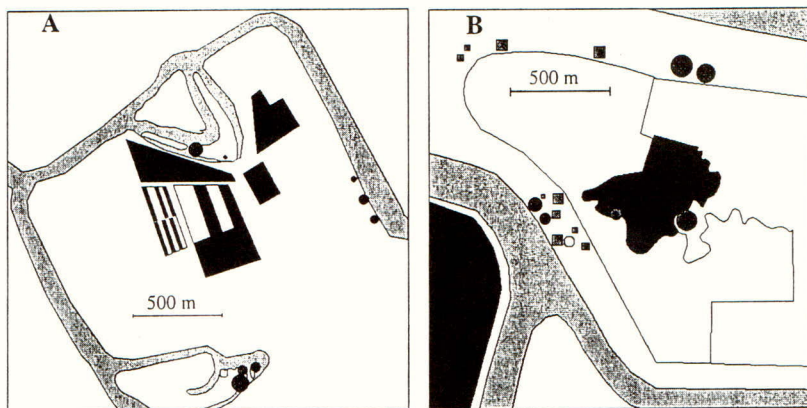
Transgene spread will depend in part on dispersal distances, establishment rates, and competitiveness of transgenic progeny relative to wild trees. For this reason we are gathering data on current levels of seed and pollen flow from hybrid poplar plantations, rates of seedling establishment in the vicinity of plantations, and competitiveness of hybrid progeny compared to wild seedlings. These studies will provide baseline gene dispersal data and allow simulation of the potential spread of transgenes in two areas of poplar cultivation in the Pacific Northwest, USA. A simulation model will serve to integrate data from field studies, information on growth and management of plantations and surrounding areas, and expected effects of the transgenes themselves on fitness. Simulation results will then be evaluated in terms of potential impacts and means for mitigation. Results from these studies will be help clarify whether stringent measures for transgene containment, such as genetically engineered sterility, are warranted.

METHODS AND MATERIALS

Study sites

We studied gene flow in the vicinity of two industrial plantations of hybrid poplar clones (*Populus trichocarpa* x *Populus deltoides*) in western Oregon: River Ranch and Clatskanie. The plantations contained large monoclonal blocks of reproductively mature male and female clones (Figure 1).

Figure 1. Study sites. A: Clatskanie; B: River Ranch. Circles represent female trees for which progeny were analyzed. Squares represent establishment plots. Symbol size represents the proportion of progeny with hybrid parentage (indicative of pollen or seed flow from plantations). ○: 0% hybrid pollination; ●: 1% hybrid pollination; ●: 3% hybrid pollination; ◐: 10% hybrid seedlings; ◑: 70% hybrid seedlings. Black blocks were mature stands of hybrid male cottonwood.



Identification of hybrid progeny

We identified progeny of hybrid parents using a three-tiered approach. First, we examined leaf morphology of seedlings to identify putative hybrids. *P. deltoides* does not occur 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 quite distinct between the parental species, and hybrid progeny are intermediate. Therefore, we used leaf morphology to screen large numbers of progeny for hybrid parentage.

Second, we confirmed hybrid parentage using molecular markers. We identified five RAPD loci (Williams *et al.*, 1990) and one simple sequence repeat locus (SSR, Powell *et al.*, 1997) that were fixed in *P. deltoides* and absent in *P. trichocarpa*. We extracted DNA from foliage and analyzed RAPDs using protocols developed in our lab (<http://www.fsl.orst.edu/tgerc/dnaext.htm>; Aagaard *et al.*, 1995). We are now performing paternity analyses on hybrid progeny using a set of highly polymorphic SSR loci developed with Dr. Toby Bradshaw, University of Washington, USA).

Gene flow by pollen

We examined pollen flow from hybrid plantations by collecting seeds from eight wild *P. trichocarpa* females growing in close proximity to the hybrid plantations (Figure 1). In order to estimate maximum expected levels of gene flow, we selected the closest female trees (2 to 500 m from plantations) that overlapped extensively in flowering phenology with hybrid clones. We germinated 100 to 300 seeds per female in a greenhouse and transplanted seedlings to the field in the spring following collection. We assessed leaf morphology following one year of growth, and extracted DNA from all putative hybrids for molecular analyses.

Gene flow by seed

We placed 20 seed traps directly adjacent to one of the plantations (River Ranch) to gauge the magnitude of hybrid gene flow by seed (Figure 1). Seeds were germinated in a greenhouse and hybrid progeny were identified as described above. We also examined seedling establishment in the vicinity of the seed traps using artificially disturbed 1 m² plots. Poplars depend on disturbance to become established; we therefore removed competing vegetation and watered during dry periods to ensure some seedling survival. We then measured the growth and survival of all seedlings, and identified hybrid progeny using RAPD and SSR markers. For comparison, we also measured growth and survival of seedlings from seed traps that had been germinated in the greenhouse and grown in randomized field plots in Corvallis, OR.

Establishment in the wild

We surveyed natural poplar establishment in the vicinity of flowering plantations in three locations: the western Columbia River near Clatskanie, Oregon; the Skagit River near Sedro Wooley, Washington; and the Fraser River west of Vancouver, British Columbia. These areas were chosen to provide the best opportunity to observe hybrid seedling establishment in the region. We measured the approximate area and density of seedling recruitment, and examined leaf morphology to identify hybrids. We also collected foliage from 96 seedlings at each site for molecular analyses.

RESULTS AND DISCUSSION

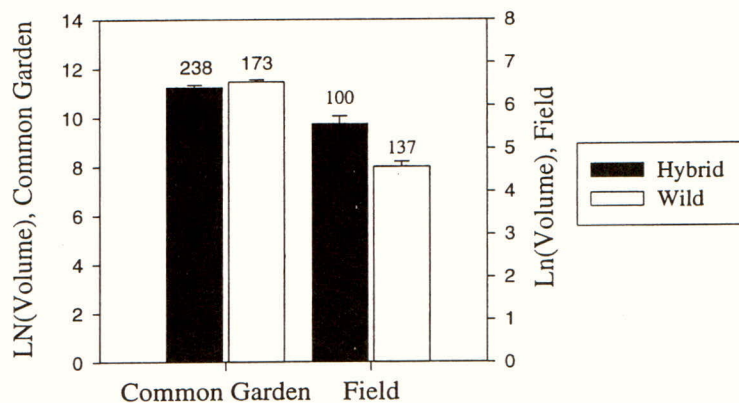
Gene flow by pollen

Assessments of hybridity based on seedling leaf morphology matched RAPD results more than 90% of the time. Frequency of hybrid progeny averaged 0.7% (range 0 to 3.6%) at Clatskanie and 1.9% (range 0 to 3.8%) at River Ranch. There was no apparent relationship with direction or distance, though the sampling was not designed to explicitly test for these effects (Figure 1).

Gene flow by seeds

On average, 18% of seedlings from seed traps were of hybrid origin (range 5 to 65%), while an average of 42% of seedlings in plots were hybrids (range 9 to 75%) (Figure 1). Overall, more hybrid seedlings established in plots than expected based on seeds collected in adjacent traps (Wilcoxon Signed-Rank Test, $P = 0.001$). Hybrids also had higher overwinter survival than wild seedlings, though the difference was not significant (Wilcoxon Signed-Rank Test, $P = 0.06$). In addition, hybrid seedlings were significantly larger than wild seedlings at the end of the growing season (ANOVA, $P = 0.0001$) (Figure 2). However, this relationship did not hold for seedlings derived from seed traps and grown under controlled conditions: there was no significant difference in size of wild and hybrid seedlings after one year of growth in the field (ANOVA, $P = 0.24$) (Figure 2).

Figure 2 Least square mean of seedling volume index (diameter² * height) of seedlings derived from seed traps and grown under controlled conditions, and seedlings naturally established in the experimental plots in the field.



Establishment in the wild

We observed a very low rate of hybrid establishment under wild conditions in the vicinity of plantations. All plantations had been flowering for at least three years prior to the survey, and one for as long as 15 years (Fraser River). However, areas of poplar establishment were relatively small, and the frequency of hybrids ranged from 0 to 1% (Table 1), despite the fact that we sampled as close to plantations as possible. These plantations were all surrounded by extensive, heavily flowering stands of wild cottonwood which may have provided a dilution effect for hybrid propagules.

Table 1. Surveys of seedling establishment in the vicinity of hybrid plantations

Site	Area (ha) ^a	Plantation Area (ha) ^b	Seedling Area (ha) ^c	Seedlings Examined	RAPD Hybrids ^d	Total Hybrids ^e
Fraser R.	100	118	0.05	~2500	1 (1%)	2
Columbia R.	2000	250	0.02	1200	1 (1%)	3
Skagit R.	56	44	0.25	>100000	0 (0%)	0

a, Approximate area surveyed for poplar establishment

b, Area of flowering hybrid poplar plantations in the survey zone

c, Approximate area of seedling establishment

d, Number (%) hybrid seedlings determined by RAPD analysis, based on 96 samples

e, Total number of hybrids, including those originally identified by leaf morphology

CONCLUSIONS

Populus trichocarpa x deltoides hybrids are capable of producing progeny that are fit enough to grow and compete in the wild in the Pacific Northwest of the United States. There is therefore little evidence that these intersectional hybrids are effectively

'domesticated.' However, because of their stringent habitat requirements, opportunities for establishment are rare. Also, the abundance of seed and pollen from wild stands may provide a powerful dilution effect, even very close to hybrid plantations. Thus, despite the physical potential for long distance gene flow from these trees, the rate of effective gene flow is likely to be very low. We will use our simulation models to explore the potential for gene spread over multiple generations. We will also examine the effects of how transgenes might affect spread rates in the presence of herbicide use or insect herbivory, or by causing male or female sterility. We will then examine the significance of these effects for wild and managed systems, including means for mitigation, if needed.

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