Finding the best trees for the job: realizing the full potential of afforestation in Canada

Robert D. Guy[a], Richard P. Pharis[b], Sally N. Aitken[a],
Ruichuan Zhang[b] and Lindsay Fung[c]

[a] *Department of Forest Sciences, 2424 Main Mall, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada*
[b] *Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada*
[c] *The Horticultural and Food Research Institute of New Zealand Ltd, Tennent Drive, Private Bag 11 030, Palmerston North, New Zealand*

Abstract

There are several million hectares of marginal agricultural lands available for afforestation in Canada. A fully implemented afforestation program could sequester as much as 28.6 megatons (Mt) of carbon per year (C/y) in above ground biomass alone. Opportunities for carbon storage or, ultimately, feedstock for biofuel production, are likely to be greatest in intensively managed stands of rapidly growing tree species. To achieve high rates of C sequestration, climate and soil conditions in our northern temperate and boreal regions favor poplar and willow. Hybrid poplars, in particular, can reach very high rates of growth. Unfortunately, most available hybrids are adapted to relatively mild climates. Our native forests, however, support four of the five native North American poplar species. Prospects for developing stress-resistant, cold-climate adapted, fast-growing hybrids or intra-species crosses are thus very good. Appropriate selections could greatly increase the potential area that can be successfully planted to poplar. Associated with afforestation using poplar will be a reduced reliance on old-growth and ancient natural forests (and the carbon already banked there) to supply wood fiber for conventional uses. Our aim is to contribute to a national afforestation effort through the development of rapid, cost-effective analyses to identify native poplar genotypes for purposes of carbon sequestration and/or biofuel production. We are investigating plant hormone profiles, carbon isotopic composition, photosynthesis, frost hardiness, and several other physiological parameters in native clones and populations of poplar and cottonwood to form a basis for selecting stock and developing breeding strategies. Experimental crosses will be field tested and successful genotypes entered into field progeny trials.

Introduction

By the early 1990's it was recognized that atmospheric CO_2 concentrations are likely to only just double if global emissions can be kept near 1990 levels. This best-case scenario became the target of the 1997 Kyoto protocol, but it still entails the likelihood of substantial climate change. All extant global circulation models predict that a doubling of atmospheric CO_2 will lead to a rise in mean temperatures of at least a few degrees Celsius (°C), and perhaps more over high latitude inland continental areas such as Canada. Although a few degrees may seem trivial, it is worth noting that the northern (tundra) and southern (grassland) boundaries of the boreal forest are separated by a difference in July mean maximum temperature of only 5°C [3]. A mere doubling of atmospheric CO_2, the international target, could therefore displace the entire boreal forest, Canada's largest and most economically important forest region.

For the year 1990, Canadian CO_2 emissions were estimated to be 162.5 megatonnes (Mt) of carbon (C). Canada's Kyoto commitment was to reduce this amount by 6% (9.75 Mt) to 152.8 Mt C by 2012. Canadian emissions were estimated to be 182.4 Mt C in 1996, already almost 30 Mt over the target level [11]. Business-as-usual projections predict emissions will reach 225 to 230 Mt C by 2020 (McIlveen 1998, cited in [12]). We clearly must urgently adopt a number of strategies to reduce net emissions. These strategies may entail, for example, increased efficiency of energy use, reduced reliance on fossil fuels through the development of alternative energy sources (wind, solar, biofuel, etc.), and enhanced uptake (sequestration) of released CO_2.

Carbon Sequestration Through Afforestation

There is considerable potential to temporarily increase carbon sequestration through afforestation and if those new forests are renewed through reforestation, the sequestration becomes "permanent". Nagle [15] estimated that approximately 7.3 million ha of marginal agricultural lands are available for afforestation in Canada. A fully implemented afforestation program could sequester as much as 28.6 Mt C per year in above-ground biomass, depending on species and area planted [9]. Even if only one million hectares (ha) were planted, something on the order of 3.9 Mt C/y could be sequestered and this is 40% of Canada's original Kyoto reduction commitment. These estimates assume a weighted mean annual stem wood volume increment of 14.6 m^3/ha/y over a 13 year weighted mean period and are in close agreement with a more detailed independent assessment by Chen et al. [4].

There is also a further increment in carbon sequestration from below-ground biomass (about 20-25%). Forest soils store large amounts of carbon and are more persistent sinks than the aboveground component [28]. Thus, afforestation (and continued reforestation) of degraded agricultural soils can improve Canada's net carbon budget irrespective of the ultimate fate of the aerial biomass.

Opportunities for carbon storage or, ultimately, feedstock for biofuel production are likely to be greatest in intensively managed stands of rapidly growing tree species. For these purposes, climate and soil conditions in temperate and boreal regions of the Northern Hemisphere favour poplar and willow [31]. Hybrid poplars, in particular, can achieve very high rates of growth; e.g., up to 54.9 t/ha over 3 years for above ground woody biomass in high-density, short rotation plantations in Washington state [5]. Assuming an oven-dry biomass to C conversion factor for hardwoods of 0.49 [19], the equivalent rate of carbon sequestration is 8.9 t/ha/y. Unfortunately, most of the available hybrid poplar genotypes are derived from species or populations adapted to relatively mild climates. While some of these "mild climate" clones are suitable to southern Ontario and southwestern British Columbia, these regions contain only a fraction of the land available for afforestation in Canada, 80% of which is located in the prairie provinces [15]. There is, however, within our native forests, a tremendous untapped genetic resource, pre-adapted to the Canadian climate.

Ignoring aspens, Canada supports four of five North American poplar species. Prospects for developing stress-resistant, cold-climate adapted, fast-growing hybrids or intra-species crosses are very high. For example, *Populus balsamifera* is found in every province, from the US border to Inuvik, while *P. trichocarpa* occurs throughout interior British Columbia and western Alberta to about 57°N latitude, and into the southwest Yukon [7]. All four Canadian species overlap in the southwest of Alberta, creating a globally unique hybridization zone. Thus, appropriate selections could greatly increase the potential land area that can be successfully planted to poplar. Associated with this, at stand maturity, will be a reduced reliance on ancient natural forests (and on carbon already banked there) to supply wood fibre for conventional uses. Indeed, van Kooten et al. [12] identified carbon storage in wood products as being essential to the economics of a national afforestation effort. Thus, the more carbon that can be directed to the bole (main stem) wood needed for "Kyoto-acceptable" products, the better.

Even a 10% increment in any of (i) growth rate, (ii) area planted, or (iii) the enhanced movement of photosynthetically fixed C from leaves into wood of the main bole, will yield tremendous value. For example, if carbon credits were to be sold at $50/t of C, a 10% increase in growth rate on a land base of 1 million ha would be worth $19.5 million/y from the increase in above-ground biomass alone.

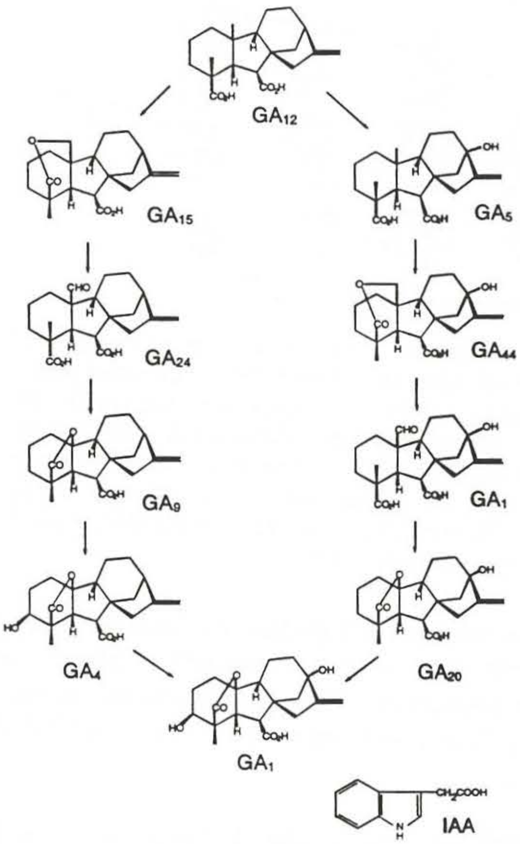

Fig. 1. Structures of key gibberellins and auxin (IAA, or indole-3-acetic acid).

The Poplar Ideotype

The ideal genotype (ideotype) for a Canadian afforestation program would combine high growth and yield with superior stress resistance and other desirable features (e.g. wood quality). Genotypes developed and planted today should also be able to take advantage of the high CO_2 world of tomorrow.

Stem growth and development of trees is regulated by plant hormones [14]. Their perception by the plant at or near the subapical and vascular cambium meristems controls stem height and diameter growth [14]. The most critical limiting hormones appear to be the gibberellins (GAs) and auxin (see Figure 1), but the other hormone classes are also involved, i.e., cytokinins, ethylene, abscisic acid, etc. Gibberellins have been implicated as a major controlling influence in hybrid vigour (heterosis) of poplar and other plants [23, 24]. Furthermore, inherently slow-growing tree genotypes/families respond disproportionately more to applied GAs (in terms of growth enhancement) than do inherently rapid-growing genotypes [see 18]. In a number of transgenic aspen lines, over expression of the GA_{20}-oxidase gene resulted in enhanced height growth and (especially) enhanced bole wood growth [6]. Even xylem fibre length was increased [6, cf 22]. Applied GAs can also modify crown form, promoting bole wood growth over branch wood growth [17].

A hypothetical example of the ideotype for crown form in an afforested crop tree is shown on the far right in Figure 2. Trees exhibiting these attributes have a high

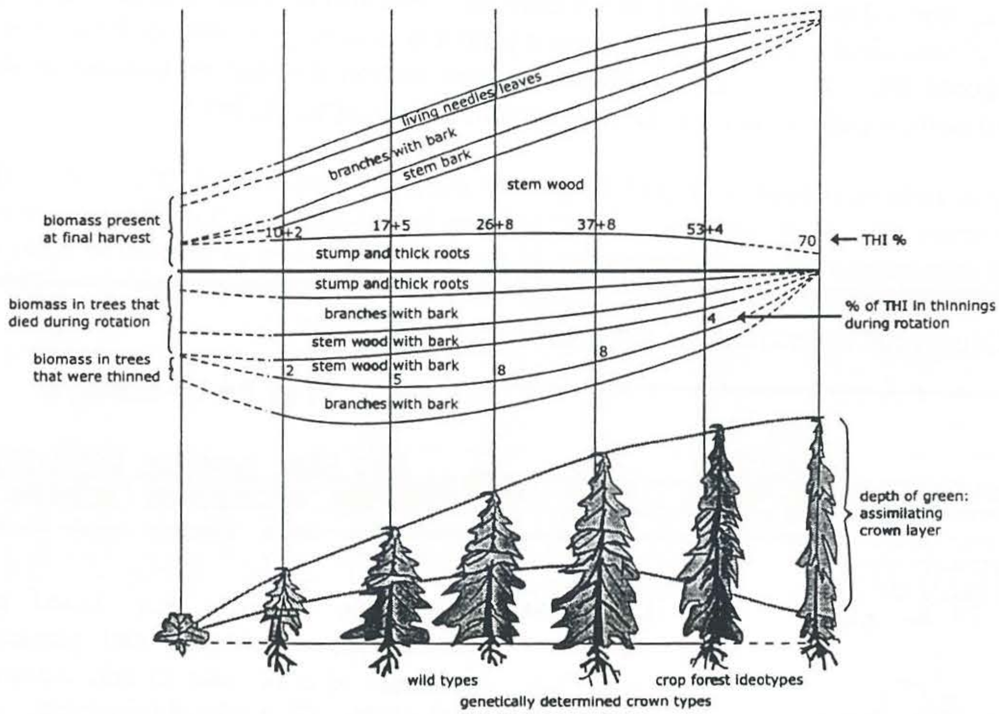

Fig. 2. Hypothetical biomass accumulation in different stem/crown form phenotypes of afforested plantation trees. Biomass in stem wood, stem bark, branch wood (including bark), leaves and stump plus thick roots is shown diagramatically. Key phenotype comparisons are "wild types" vs "crop forest ideotypes". THI = Total Harvest Index: the share of valuable stem wood as a proportion of the total above and belowground biomass. The narrow crowns of crop forest ideotypes permit a higher number of stems per hectare and a "deeper" layer where photoassimilation remains active, resulting in an enhanced THI. Adapted from the 1985 Annual Report of the Foundation for Forest Tree Breeding in Finland, p. 125.

proportion of stem wood relative to branch wood (i.e., a high Harvest Index). They also permit higher stand densities (more stems per hectare) and reductions in woody debris from branches that would be destined for premature litter decomposition during the rotation cycle. Several studies for conifers [27, 30] and one study for poplars [29] show that Harvest Index is a strongly inherited trait.

The main problem with conventional high-yielding poplar hybrids is inadequate adaptation to the length and severity of the Canadian winter. The degree of cold hardiness, and thus the risk of cold injury, in temperate and boreal tree species is largely a function of the timing of the annual developmental cycle rather than the maximum level of cold hardiness attained. The challenge for breeding is to maintain the synchronization of growth and dormancy across a range of planting site, while increasing carbon fixation. In principle, greater biomass accretion may result from either a higher intrinsic growth rate (i.e., over a fixed season) or from a longer period of growth (i.e., an average growth rate, but one that is extended over a longer season).

Negative genetic correlations frequently exist between total growth and cold hardiness across populations for temperate and boreal conifers, and the same is likely true for hardwoods [1]. These unfavorable relationships often result when one uses populations with higher inherent growth potential, likely because the populations originate from, and are adapted to, longer growing seasons. Similar relationships may or may not be found within populations, depending on whether correlations are a function of pleiotropy or linkage disequilibrium. Thus, selection on growth alone may increase the risk of cold injury. However, if genotypes are selected for maximum growth rate rather than total growth, genotypes can be found that complete more growth within the period of low frost risk, rather than completing more growth at the risk of increased cold injury [20]. Hence, profiling hormones at the time of maximum growth rate may be informative in identifying clones with the most rapid growth during the frost-free months, as opposed clones that merely have a longer period of fast growth.

Another problem with conventional hybrids demand large quantities of water and nitrogen, severely limiting deployment on the prairies. When levels of these resources vary in the environment, plant water-use efficiency (WUE) and nitrogen-use efficiency (NUE) trade-off such that an increase in one incurs a decrease in the other [16]. However, such a trade-off is not necessarily associated with genetic variation. Thus, among populations of lodgepole pine (Smets et al., in prep), NUE and stable carbon isotope ratio ($\delta^{13}C$; an excellent proxy for WUE) correlate negatively when nitrogen islimiting and positively when it is sufficient. We think that the N-limiting situation may reflect inherent or environmental differences in ability to acquire nitrogen, whereas the positive correlation under N-sufficiency appears to reflect <u>inherent variation in growth rate</u>. We have thus found for several conifer tree species that genetic variation in $\delta^{13}C$ is consistently correlated not only with WUE, but also with growth potential (e.g. Figure 3), i.e. inherent growth rate under "reasonable" environmental conditions [10, 25, 26].

Preliminary results (data not shown) show a similar relationship for two woody angiosperms, Sitka alder and an intra-specific cross of *P. deltoides*. Higher WUE in combination with superior growth means increased photosynthesis, not reduced transpiration, and this has been confirmed through gas exchange analysis. Such a relationship should be expected wherever the rate of utilization of photosynthate (i.e. strength of the final "sink" for carbon) controls photosynthesis. Hence, we believe that there is a significant potential to use $\delta^{13}C$ values to simultaneously select for enhanced growth as well as NUE and WUE. Finally, we should note that for *Pinus* seedlings, both growth and WUE can be promoted by a soil application of the gibberellin $A_{4/7}$ mixture (Table 1).

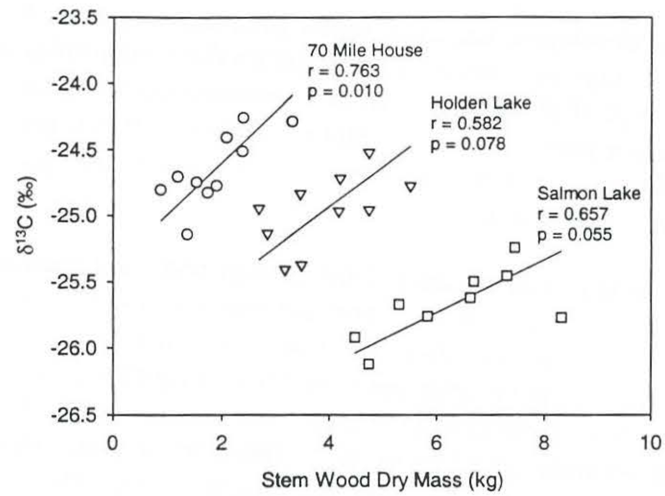

Fig 3. Correlations between mean $\delta^{13}C$ and stem wood dry mass for 10 populations of *Pinus contorta* growing at three test sites in British Columbia. In most cases, each point represents mean of 18 trees. Error bars omitted for clarity. Adapted from Guy & Holowachuk [10]. Stem wood dry mass was calculated from volume based on a green wood density of 0.406 [8].

Table 1. Effect of soil drenching with a mixture of gibberellins A_4 and A_7 on mean height and stem dry weight (DW) increments, relative to controls, and $\delta^{13}C$ of six greenhouse-grown lodgepole pine half-sib families. Isotope analysis was performed on terminal buds after primary growth cessation. Both standard deviation (±SD) and standard error (±SE) are shown. Higher (less negative) $\delta^{13}C$ following treatment with $GA_{4/7}$ indicates higher water-use efficiency commensurate with enhanced height and stem DW growth. Mean $\delta^{13}C$ values for control and $+GA_{4/7}$ treated families differ significantly at p=0.002 (paired t-test).

	% Height increment induced by $GA_{4/7}$	**% Stem DW increment induced by $GA_{4/7}$**	**Control $\delta^{13}C$ (‰)**	**$+GA_{4/7}$ $\delta^{13}C$ (‰)**	**$\Delta\ \delta^{13}C$ (control - $GA_{4/7}$)**
Mean (n=6)	+26.7	+73.3	-28.02	-27.32	0.71
±SD	12.6	26.0	0.39	0.50	0.28
±SE	5.2	10.6	0.16	0.20	0.12

In summary, the poplar ideotype should:

a. have a fast growth rate, within the limitations imposed by the regional environment.
b. be adapted to short summers and the risk of growing-season frost.
c. be winter hardy to temperatures as low as -50°C.
d. be drought resistant, especially for prairie/parkland afforestation.
e. be insect and disease resistant.
f. have an optimum form (large bole, fine branches) for maximizing carbon storage in the bole wood during the life of the tree.
g. have high WUE and high NUE.
h. be highly growth responsive to increasing atmospheric CO_2.

There is a need for several separate lines having most, if not all, of the traits identified above. The evidence in hand indicates that one should be able to identify phenotypic markers for these properties, and ultimately select and/or develop novel, stress resistant lines of poplar appropriate to the target regions of Canada. The probability of success in terms of locating promising material in the natural gene pool, and generating new hybrids through crossing, is very high (poplars are relatively easy to breed).

Clone Deployment and Biodiversity

Short-rotation, fast-growing hardwoods such as hybrid poplars are typically grown in single-species, even-aged plantations. It is difficult to grow these trees in multi-species mixtures as the early, rapid rate of growth of the poplars will shade out slower-growing species such as white spruce. Large-scale industrial plantations or fibre farms, such as those on private lands in Washington and Oregon, are planted as mosaics of monoclonal blocks. This increases product uniformity and simplifies deployment and harvest compared to intimate mixtures of clones. However, clonal blocks may be unacceptable for some areas in Canada due to a lack of structural or visual diversity. Smaller clonal blocks or clonal mixtures may thus be desirable if C sequestration plantations have multiple additional objectives. Numbers of clones deployed, whether in blocks or intimate mixtures, is related in a non-linear fashion to both risk and genetic gain and will require careful consideration [13].

Objectives

Our long-term objective is to develop rapid, and cost-effective phenotypic analyses that can be used in practice to identify suitable poplar genotypes (or characteristics to combine into genotypes) from existing clonal material and from new clones derived from controlled crosses. Our short-term objectives for native clones/populations of *P. trichocarpa* and *P. balsamifera* are to:

1. characterise patterns in growth potential, photosynthetic capacity (including sink strength of the bole), water and nitrogen-use efficiencies and frost hardiness.
2. characterise changes in patterns and levels of phytohormones that are responsible for stem biomass accumulation thereby forming a basis for selecting stock and developing breeding strategies.
3. perform experimental crosses to test our hypotheses, with subsequent entry of the progeny into field trials.
4. examine genetic correlations between above-ground biomass (especially bole wood) and total plant C sequestration, and delineate tradeoffs between carbon fixation and stress resistance in different environments.

Recent Progress

Within the last eight months we have:

1. begun validating and improving methods for phytohormone analysis of main bole and branch cambial region tissues from inherently fast- and slow-growing clones (genotypes) of poplar,

2. initiated the process of correlating endogenous hormone levels with inherent growth capacity,
3. assessed $\delta^{13}C$ values of wood from these same stems, and
4. assessed the growth period, photosynthetic rates, frost hardiness and $\delta^{13}C$ in a limited selection of available native *P. trichocarpa* clones.

Significant progress has been made on all fronts.

In collaboration with the Poplar Afforestation Program at HortResearch, in New Zealand, we harvested cambial region tissues from actively growing trees in a field progeny trial in Palmerston North. Concentrations of several GAs, ABA and IAA were determined by Gas Chromatography-Mass Spectrometry-Selected Ion Monitoring (GC-MS-SIM) using isotope dilution techniques of stable isotope-labeled hormones. These results were obtained "retrospectively" in a blind fashion (Table 2) and confirm earlier bioassay results using *P. deltoides* interspecific hybrids (Table 3 and [23]). We have also made a preliminary assessment of endogenous GAs in cambial region tissues of two poplar hybrids, one slow- and one fast-growing, by GC-MS-SIM and the fast-growing hybrid showed elevated GAs (Table 2). Recently, Eriksson et al. [6] examined transgenic lines of hybrid aspen where a GA biosynthetic enzyme (GA 20 oxidase) was being over expressed. The transgenic lines were overproducing "growth-effector" GAs from C_{20} GAs earlier in the GA biosynthetic pathway (Table 4), yielding a concomitant reduction in endogenous C_{20} GAs. Interestingly, in the fast-growing genotype that we examined (Table 2), there are large amounts of both C_{20} GAs (precursors in the GA biosynthetic pathway) and the growth-effector GA, GA_1 (see Figure 1). These three examples thus

Table 2. Levels of endogenous gibberellins (GA), abscisic acid (ABA) and indole-3-acetic acid (IAA) from cambial region tissue scrapings (35 to 90 mg dw) of 7-yr-old *Populus deltoides* × *P. ciliata* (G5) and *P. deltoides* × *P. szechuanica* (E6) genotypes. These are preliminary assessments of trees grown in a field progeny trial at Palmerston North, NZ by the HortResearch Crown Research Institute. Samples were taken 5 weeks after leaves had reached full size. Hormone quantification was based upon the dilution of stable isotope internal standards of $[^2H_2]$ GAs (8-10 ng), $[^2H_6]$-ABA (100 ng) and $[^{13}C_6]$ IAA (100 ng) by the endogenous, unlabelled hormone using GC-MS-SIM.

	G5 (fast-grower)		**E6 (slow-grower)**	
	ng/g DW scraped tissue	**pg/cm^2 scraped area**	**ng/g DW scraped tissue**	**pg/cm^2 scraped area**
GA_1	22.6	17.0	Trace	Trace
GA_{15}	36.6	27.6	16.1	4.57
GA_{19}	90.6	68.2	60.7	17.22
GA_{44}	24.1	18.1	19.3	5.47
ABA	278	209	774	220
IAA	27,000	20,321	64,000	18,157

Trace = less than 0.1 ng present in tissue sample

Note: Based on the presence of GA_{19} and GA_{44} these tissues are presumed to possess the early 13-hydroxylation pathway of gibberellin biosynthesis. Similarly, the presence of GA_{15} implies that the early non-hydroxylation pathway is also present.

Table 3. Levels of gibberellin (GA)-like substances in cambial region scrapings from stem internodes of poplar as determined by the single gene dwarf-rice, cv Tan-ginbozu bioassay. Adapted from Rood & Pharis [23].

	Clone	GA-like activity ($g\ GA_3$ equiv / g DW tissue)
Populus deltoides		
	D36	0.47 ± 0.23[a]
	D56	0.61 ± 0.27
P. deltoides × *P. nigra* (rapid growing interspecific hybrids)	DN 138[b]	0.79 ± 0.34
	DN 103	1.23 ± 0.55
	DN 128	2.32 ± 0.42
	DN 160	5.66 ± 1.89

[a] Mean ±SE. Values presented are total of gibberellin-like bioactivity of all chromatographic regions combined.
[b] D36 was a parent of DN 103 and DN 160, while D56 was a parent of the other hybrids

provide good evidence that inherently fast-growth in poplar and aspen species, at least, is causally correlated with elevated GA levels and/or biosynthetic turnover.

We have begun a phenotypic assessment of native *P. trichocarpa* genotypes from a BC Ministry of Forests (BCMoF) collection of some 835 clones. These clones come from all over BC, including the central interior and the Cariboo plateau. They are being maintained in stool beds at Surrey, Terrace and Cowichan Lake. In spring 2000, 4200 stems originating from these clones were planted into five blocks in a common garden at the Surrey Nursery under the direction of Dr. Cheng Ying (BCMoF). We have chosen a subset of 25 clones (14 from interior BC, seven from coastal BC, two from Washington State, one from Oregon and one from Alaska) for analysis of isotopic composition, photosynthetic rates and frost hardiness development. This research is ongoing.

Table 4. The influence of GA 20-oxidase expression (*AtGA20ox1*) in two transgenic lines of hybrid aspen (*Populus tremula* × *P. tremuloides*), relative to the wild-type hybrid aspen control. The multifunctional GA 20-oxidase enzyme catalyzes the stepwise conversion of C_{20} gibberellins (GA_{12}/GA_{53}) to C_{19} gibberellins (GA_9\GA_{20}), the latter being the immediate 3-deoxy precursors to the growth-effector, 3ß-hydroxylated GAs, GA_4 and GA_1, respectively. Adapted from Eriksson et al. [6].

	Average internode length (cm ±SE)[a]	Stem dry mass (g ±SE)	Stem GA_4 levels[a] (ng/g fresh wt)	Stem GA_1 levels (ng/g fresh wt)
Control	2.19 ± 0.05	4.58 ± 0.51	0.84	0.63
Transgenic Line 2	3.06 ± 0.09	10.42 ± 1.01	9.04	10.2
Transgenic Line 11	3.07 ± 0.09	10.34 ± 0.49	10.7	14.2

[a]internodes 7 and 8

Our Future Approach

We hope to expand our utilization of the available *P. trichocarpa* clones to assess population level (rather than just clonal) differences in $\delta^{13}C$, frost hardiness and other phenotypic variables. Ultimately, these data will be subjected to multiple regression or canonical correlation analysis against provenance location and climatic variables to discern geographic trends [2]. Appropriate climatic models are now becoming more widely available [e.g., 21]. Through multi-factor analysis of physiological traits, adaptive patterns of variation in terms of specific ecophysiological traits and underlying abiotic selective agents can be better elucidated. We will also expand our investigations to include collections of *P. balsamifera* and other material made available to us by an industrial collaborator (Alberta Pacific Forest Industries Ltd.). These collections will be supplemented by additional material obtained from the wild. Eventually, hybrids of these two species will be produced by us and by our non-academic participants.

The use of "retrospective" tests is a very important tool in our future research. It will allow us to examine correlations between growth, $\delta^{13}C$ and phytohormone profiles using established hybrid trials from New Zealand, British Columbia, Alberta, and eventually from other regions of Canada. In such retrospective examinations we will identify a range of clones differing in crown form (i.e. bole size and relative proportion of branch wood) and stem biomass production. These genotypes will be utilised for sampling of cambial tissues and adjacent branch and stem wood for analysis of endogenous hormone levels and $\delta^{13}C$, respectively.

We envision making substantial progress in breeding programs within 5 years, but will need early on to identify and collect promising germplasm from nature and also from existing collections to establish baseline populations. Although 2-3 years will be needed to bulk material up before genecological testing of new accessions can begin (and likely 5+ years before first generation hybrids are available), testing of currently available clones will begin this coming year for selection as parental lines and for retrospective evaluation of early-progeny testing.

In tree improvement, the ecological conditions to which genotypes are suitably adapted have typically been evaluated in long-term field trials. Increasingly, however, genotypes are being assessed with young plants in controlled, common garden experiments under a variety of treatments. In this way, the genetic variation among populations or clones, the factors that result in Genotype $\times$ Environment (G$\times$E) interactions and the range of conditions across which performance is stable can be quantified fairly rapidly. Large raised beds are well suited to such experiments and will be established in at least two locations (UBC campus and a second site yet to be identified). Initially, norms of response for genotypes of parental species, as well as a standard set of existing "mild climate" clones, will be characterised. First generation hybrids will be included as they are produced. The raised beds will also be used to examine genetic correlations between aboveground biomass and total plant C sequestration, and tradeoffs between carbon fixation and stress resistance in different environments.

Phytohormone analysis will be accomplished by GC-MS-SIM (see above). Using this technique we can determine the proper/optimal time for cambial region tissue sampling; e.g., during the log phase of wood growth, etc. Cambial region material (xylem and phloem) will be sampled about 7 to 8 weeks into active xylem growth/differentiation. Use of the cambial region tissues is likely to be less variable than attempting to "capture" elongating internodes of a range of genotypes at a consistent stage of ontogeny. For $\delta^{13}C$ isotopic analysis, leaves, petioles or wood will be stored in liquid N_2 until oven drying. Analysis can be accomplished on as little as 1 mg of the dried tissue, and these subsamples will be analysed for $\delta^{13}C$ using a model 1106 Elemental Analyser interfaced to a Prism triple-collecting ratio mass spectrometer. An advantage of this system is that it also yields the N content, which in conjunction with determinations of net photosynthetic rate can provide information on nitrogen-use efficiency.

In summary, we believe that it will be possible to develop both time- and cost-effective methodse that will allow for the early identification of inherently fast-growing genotypes of poplar and aspen that are also insect, disease and stress resistant. We expect these genotypes to originate from inter- and intra-specific crosses and to include individuals that exhibit the classic definition of hybrid vigour.

Literature Cited

1. Aitken, SN, M Hannerz. 2001. Genecology and gene resource management strategies for conifer cold hardiness. *In* Conifer Cold Hardiness. FJ Bigras, SJ Colombo (eds). Kluwer Academic Publishers, Dordrecht. pp 23-53
2. Benowicz, A, RD Guy, YA El-Kassaby. 2000. Geographic pattern of genetic variation in photosynthetic capacity and growth in two hardwood species from British Columbia. Oecologia 123:168-174
3. Bonan, GB, D Pollard, SL Thompson. 1992. Effects of boreal forest vegetation on global climate. Nature 359:716-718
4. Chen, W, JM Chen, DT Price, J Cihlar, J Liu. 2000. Carbon offset potentials of four alternative forest management strategies in Canada: a simulation study. Mitigation and Adaptation Strategies for Global Change 5:143-169
5. DeBell, DS, CA Harrington. 1997. Productivity of *Populus* in monoclonal and polyclonal blocks at three spacings. Can J For Research 27:978-985
6. Eriksson, ME, M Israelsson, O Olsson, T Moritz. 2000. Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. Nature Biotech 18:784-788
7. Farrar, JL. 1995. Trees in Canada. Fitzhenry & Whiteside, Markham. 502 pp
8. Gonzales, JS. 1990. Wood density of Canadian tree species. Forestry Canada, Northwest Region, Northern Forestry Centre, Edmonton. Information Report NOR-X-315
9. Guy, RD, A Benowicz. 1998. Can afforestation contribute to a reduction in Canada's net CO_2 emissions? Report to Canadian Pulp & Paper Association. 17 pp
10. Guy, RD, DL Holowachuk. 2001. Population differences in stable carbon isotope ratio of *Pinus contorta* Dougl. ex Loud.: relationship to environment, climate of origin and growth potential. Can J Bot 79:274-283
11. Jacques, A. 1998. Revised 1990 and 1996 greenhouse gas emissions estimates. Env Canada, Pollution Data Branch, Ottawa
12. van Kooten, GC, E Krcmar-Nozic, B Stennes, R van Gorkum 1999. Economics of fossil fuel substitution and wood product sinks when trees are planted to sequester carbon on agricultural lands in western Canada. Can J For Research 29:1669-1678.

13. Libby, WJ. 1982. What is a safe number of clones per plantation? *In* Resistance to Diseases and Pests in Forest Trees. HM Heybroek, BR Stephen, K von Weissenberg (eds). Proc Third International Workshop on Genetics of Host-parasite Interactions in Forestry, Wageningen. pp 342-360
14. Little, CHA, RP Pharis. 1995. Hormonal control of radial and longitudinal growth in the tree stem. *In* Plant Stems: Physiology and Functional Morphology. B Gartner (ed). Academic Press, New York. pp 281-319
15. Nagle, GS. 1990. Technical Background Paper on Trees in Canada Program. *Prepared for* Forestry Canada *by* Nawitka Renewable Resource Consultants Ltd, Victoria. 68 pp
16. Patterson, TB, RD Guy, QL Dang. 1997. Whole-plant nitrogen- and water-relations traits, and their associated trade-offs, in adjacent muskeg and upland boreal spruce species. Oecologia 110:160-168
17. Pharis, RP. 1976. Probable roles of plant hormones in regulating shoot elongation, diameter growth and crown form in conifers. *In*: Physiological Genetics of Forest Trees. M Cannell, F Last (eds). Academic Press, London. pp 291-306
18. Pharis, RP, R Zhang, IB-J Jiang, BP Dancik, FC Yeh. 1992. Differential efficacy of gibberellins in flowering and vegetative shoot growth, including heterosis and inherently rapid growth. *In*: Progress in Plant Growth Regulation, CM Karssen, LC Van Loon, D Vreugdenhil (eds). Kluwer, Amsterdam. pp 13-27
19. Ragland, KW, DJ Aerts, AJ Baker. 1991. Properties of wood for combustion analysis. Bioresource Technology 37:161-168
20. Rehfeldt, GE. 1992. Early selection in *Pinus ponderosa*: compromises between growth potential and growth rhythm in developing breeding strategies. For Sci 38:661-677
21. Rehfeldt, GE, CC Ying, DL Spittlehouse, DA Hamilton, Jr. 1999. Genetic responses to climate in *Pinus contorta*: niche breadth, climate change and reforestation. Ecol Monogr 69:375-407
22. Ridoutt, BG, RP Pharis, R Sands. 1996. Secondary xylem fibre development and levels of cambial region gibberellins A_1 and A_{20}, indole-3-acetic acid and abscisic acid in *Eucalyptus globulus* following stem injection of an acyclohexanedione derivative known to inhibit gibberellin biosynthesis. Physiol Plant 96:559-566
23. Rood, SB, RP Pharis 1987. Hormones and heterosis in plants. *In* Plant Hormones and their Role in Plant Growth and Development. PC Davies (ed). Agric Biotech Series, Martinus Nijhoff/Junk, Dordrecht. pp 463-473
24. Rood, SB, RI Buzzell, LN Mander, D Pearce, RP Pharis. 1988. Gibberellins: A phytohormonal basis for heterosis in maize. Science 241:1216-1218
25. Silim, SN, RD Guy, TB Patterson, NJ Livingston. 2001. Plasticity in water-use efficiency of *Picea sitchensis*, *P. glauca* and their natural hybrids. Oecologia 128:317-325
26. Sun, ZJ, NJ Livingston, RD Guy, GJ Ethier. 1996. Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (*Picea glauca* (Moench) Voss) seedlings. Plant Cell Environ 19:887-894
27. Tigerstedt, PMA. 1969. Progeny test in a *Pinus sylvestris* L. seed orchard in Finland. Acta For Fenn 99:1-17
28. Thuille, A, N Buchmann, ED Schulze 2000. Carbon stocks and soil respiration rates during deforestation, grassland use and subsequent Norway spruce afforestation in the Southern Alps, Italy. Tree Physiol 20:849-857
29. Wu, R, R Stettler. 1998. Quantitative genetics of growth and development in *Populus*. III. Phenotypic plasticity of crown structure and function. Heredity 81:299-310
30. Wu, HXM, FC Yeh. 1997. Genetic effect on biomass partition and breeding for tree architecture in *Pinus contorta* spp. *latifolia* from central Alberta. For Genet 4:123-131
31. Zsuffa, L, E Giordano, LD Pryor, RF Stettler. 1996. Trends in poplar culture: some global and regional perspectives. *In* Biology of *Populus* and its Implications for Management and Conservation. RF Stettler, HD Bradshaw Jr, PE Heilman, TM Hinckley (eds). NRC Research Press, Ottawa. pp 515-537