

The outcomes from this project are summarized in 7 peer reviewed publications that are listed below with their respective abstracts.

1. Elias, Ani A., Victor B. Busov, Kevin R. Kosola, Cathleen Ma, Elizabeth Etherington, Olga Shevchenko, Harish Gandhi, David W. Pearce, Stewart B. Rood, and Steven H. Strauss. (2012) Green revolution trees: Semi-dwarfism transgenes modify gibberellins, promote root growth, enhance morphological diversity, and reduce competitiveness in *Populus*. *Plant Physiol.* 160: 1130-1144

Semidwarfism has been used extensively in row crops and horticulture to promote yield, reduce lodging, and improve harvest index, and it might have similar benefits for trees for short-rotation forestry or energy plantations, reclamation, phytoremediation, or other applications. We studied the effects of the dominant semidwarfism transgenes GA Insensitive (GAI) and Repressor of GAI-Like, which affect gibberellin (GA) action, and the GA catabolic gene, GA 2-oxidase, in nursery beds and in 2-year-old high-density stands of hybrid poplar (*Populus tremula* × *Populus alba*). Twenty-nine traits were analyzed, including measures of growth, morphology, and physiology. Endogenous GA levels were modified in most transgenic events; GA(20) and GA(8), in particular, had strong inverse associations with tree height. Nearly all measured traits varied significantly among genotypes, and several traits interacted with planting density, including aboveground biomass, root-shoot ratio, root fraction, branch angle, and crown depth. Semidwarfism promoted biomass allocation to roots over shoots and substantially increased rooting efficiency with most genes tested. The increased root proportion and increased leaf chlorophyll levels were associated with changes in leaf carbon isotope discrimination, indicating altered water use efficiency. Semidwarf trees had dramatically reduced growth when in direct competition with wild-type trees, supporting the hypothesis that semidwarfism genes could be effective tools to mitigate the spread of exotic, hybrid, and transgenic plants in wild and feral populations.

2. Zawaski C, Kadmiel M, Pickens, J, Ma C, Strauss SH, and Busov VB (2011) Repression of gibberellin biosynthesis or signaling produces striking alterations in poplar growth, morphology, and flowering. *Planta* 234:1285-98

We modified gibberellin (GA) metabolism and signaling in transgenic poplars using dominant transgenes and studied their effects for 3 years under field conditions. The transgenes that we employed either reduced the bioactive GAs, or attenuated their signaling. The majority of transgenic trees had significant and in many cases dramatic changes in height, crown architecture, foliage morphology, flowering onset, floral structure, and vegetative phenology. Most transgenes elicited various levels of height reduction consistent with the roles of GA in elongation growth. Several other growth traits were proportionally reduced, including branch length, internode distance, and leaf length. In contrast to elongation growth, stem diameter growth was much less affected, suggesting that semi-dwarf trees in dense stands might provide high levels of biomass production and carbon sequestration. The severity of phenotypic effects was strongly correlated with transgene expression among independent transgenic events, but

often in a non-linear manner, the form of which varied widely among constructs. The majority of semi-dwarfed, transgenic plants showed delayed bud flush and early bud set, and expression of a native GAI transgene accelerated first time flowering in the field. All of the phenotypic changes observed in multiple years were stable over the 3 years of field study. Our results suggest that transgenic modification of GA action may be useful for producing semi-dwarf trees with modified growth and morphology for horticulture and other uses.

3. Gou, J., Ma C, Kadmiel M, Gai Y, Strauss SH, Jiang X, and Busov VB (2011) Tissue-specific expression of *Populus* C₁₉ GA 2-oxidases differentially regulate above and below ground biomass growth through control of bioactive GA levels. *New Phytologist* 192: 626–639

We studied the poplar C(19) gibberellin 2-oxidase (GA2ox) gene subfamily. We show that a set of paralogous gene pairs differentially regulate shoot and root development. • PtGA2ox4 and its paralogous gene PtGA2ox5 are primarily expressed in aerial organs, and overexpression of PtGA2ox5 produced a strong dwarfing phenotype characteristic of GA deficiency. Suppression of PtGA2ox4 and PtGA2ox5 led to increased biomass growth, but had no effect on root development. By contrast, the PtGA2ox2 and PtGA2ox7 paralogous pair was predominantly expressed in roots, and when these two genes were RNAi-suppressed it led to a decrease of root biomass. • The morphological changes in the transgenic plants were underpinned by tissue-specific increases in bioactive GAs that corresponded to the predominant native expression of the targeted paralogous gene pair. Although RNAi suppression of both paralogous pairs led to changes in wood development, they were much greater in the transgenics with suppressed PtGA2ox4 and PtGA2ox5. The degree of gene suppression in independent events was strongly associated with phenotypes, demonstrating dose-dependent control of growth by GA2ox RNA concentrations. • The expression and transgenic modifications reported here show that shoot- and leaf-expressed PtGA2ox4 and PtGA2ox5 specifically restrain aerial shoot growth, while root-expressed PtGA2ox2 and PtGA2ox7 promote root development.

4. Zawaski C, Kadmiel M, Ma C, Gai Y, Jiang X, Strauss SH, and Busov VB (2011) *SHORT INTERNODES*-like genes regulate shoot growth and xylem proliferation in *Populus*. *New Phytologist*. 191: 678–691

Genes controlling plant growth and form are of considerable interest, because they affect survival and productivity traits, and are largely unknown or poorly characterized. The *SHORT INTERNODES*(SHI) gene is one of a 10-member SHI-RELATED SEQUENCE (SRS) gene family in *Arabidopsis* that includes important developmental regulators. • Using comparative sequence analysis of the SRS gene families in poplar and *Arabidopsis*, we identified two poplar proteins that are most similar to SHI and its closely related gene *STYLISH1* (STY1). The two poplar genes are very similar in sequence and expression and are therefore probably paralogs with redundant functions. • RNAi suppression of the two *Populus* genes enhanced shoot and root growth, whereas the overexpression of *Arabidopsis* SHI in poplar reduced internode and petiole length. The suppression of the two genes increased fiber length and the proportion of xylem tissue, mainly through increased xylem cell proliferation. The transgenic modifications were also associated with significant changes in the concentrations of gibberellins and cytokinin. • We

conclude that *Populus* SHI-RELATED SEQUENCE (SRS) genes play an important role in the regulation of vegetative growth, including wood formation, and thus could be useful tools for the modification of biomass productivity, wood quality or plant form.

5. Katherine M. Han, Palitha Dharmawardhana, Renee´ S. Arias, Cathleen Ma, Victor Busov and Steven H. Strauss. (2010) Gibberellin-associated cisgenes modify growth, stature and wood properties in *Populus*. *Plant Biotech J.* p 1–17.

We studied the effects on plant growth from insertion of five cisgenes that encode proteins involved in gibberellin metabolism or signalling. Intact genomic copies of PtGA20ox7, PtGA2ox2, PtRGL1_1, PtRGL1_2 and PtGAI1 genes from the genome-sequenced *Populus trichocarpa* clone Nisqually-1 were transformed into *Populus tremula* × *alba* (clone INRA 717-1B4), and growth, morphology and xylem cell size characterized in the greenhouse. Each cisgene encompassed 1-2 kb of 5' and 1 kb of 3' flanking DNA, as well as all native exons and introns. Large numbers of independent insertion events per cisgene (19-38), including empty vector controls, were studied. Three of the cisgenic modifications had significant effects on plant growth rate, morphology or wood properties. The PtGA20ox7 cisgene increased rate of shoot regeneration in vitro, accelerated early growth, and variation in growth rate was correlated with PtGA20ox7 gene expression. PtRGL1_1 and PtGA2ox2 caused reduced growth, while PtRGL1_2 gave rise to plants that grew normally but had significantly longer xylem fibres. RT-PCR studies suggested that the lack of growth inhibition observed in PtRGL1_2 cisgenic plants was a result of co-suppression. PtGAI1 slowed regeneration rate and both PtGAI1 and PtGA20ox7 gave rise to increased variance among events for early diameter and volume index, respectively. Our work suggests that cisgenic insertion of additional copies of native genes involved in growth regulation may provide tools to help modify plant architecture, expand the genetic variance in plant architecture available to breeders and accelerate transfer of alleles between difficult-to-cross species.

6. Gou, J., Strauss, S.H., Tsai, C.J., Fang, K., Chen, Y., Jiang, X., and Busov, V.B. (2010) Gibberellins Regulate Lateral Root Formation in *Populus* through Interactions with Auxin and Other Hormones. *Plant Cell* 22:623-639.

The role of gibberellins (GAs) in regulation of lateral root development is poorly understood. We show that GA-deficient (35S:PcGA2ox1) and GA-insensitive (35S:rgl1) transgenic *Populus* exhibited increased lateral root proliferation and elongation under in vitro and greenhouse conditions, and these effects were reversed by exogenous GA treatment. In addition, RNA interference suppression of two poplar GA 2-oxidases predominantly expressed in roots also decreased lateral root formation. GAs negatively affected lateral root formation by inhibiting lateral root primordium initiation. A whole-genome microarray analysis of root development in

GA-modified transgenic plants revealed 2069 genes with significantly altered expression. The expression of 1178 genes, including genes that promote cell proliferation, growth, and cell wall loosening, corresponded to the phenotypic severity of the root traits when transgenic events with differential phenotypic expression were compared. The array data and direct hormone measurements suggested crosstalk of GA signaling with other hormone pathways, including auxin and abscisic acid. Transgenic modification of a differentially expressed gene encoding an auxin efflux carrier suggests that GA modulation of lateral root development is at least partly imparted by polar auxin transport modification. These results suggest a mechanism for GA-regulated modulation of lateral root proliferation associated with regulation of plant allometry during the stress response.

7. Busov VB, Brunner AM, Strauss SH. 2008. Genes for control of plant stature and form. *New Phytologist* 177: 589-607.

Here we summarize progress in identification of three classes of genes useful for control of plant architecture: those affecting hormone metabolism and signaling; transcription and other regulatory factors; and the cell cycle. We focus on strong modifiers of stature and form that may be useful for directed modification of plant architecture, rather than the detailed mechanisms of gene action. Gibberellin (GA) metabolic and response genes are particularly attractive targets for manipulation because many act in a dose-dependent manner; similar phenotypic effects can be readily achieved in heterologous species; and induced pleiotropic effects--such as on nitrogen assimilation, photosynthesis, and lateral root production--are usually positive with respect to crop performance. Genes encoding transcription factors represent strong candidates for manipulation of plant architecture. For example, AINTEGUMENTA, ARGOS (auxin-regulated gene controlling organ size), and growth-regulating factors (GRFs) are strong modifiers of leaf and/or flower size. Plants overexpressing these genes had increased organ size and did not display negative pleiotropic effects in glasshouse environments. TCP-domain genes such as CINCINNATA, and the associated regulatory miRNAs such as miRJA, may provide useful means to modulate leaf curvature and other foliage properties. There are considerable opportunities for comparative and translational genomics in nonmodel plant systems.