‘Plant hormone markers for inherently rapid stem diameter & height growth in balsam poplar and hybrid poplar genotypes growing in Canada’s Prairie Provinces’

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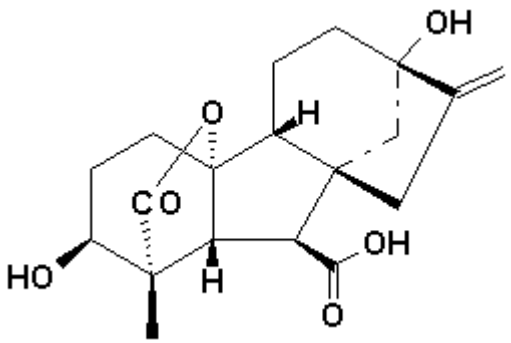
New Zealand's "famous" 80055 genotype of *Pinus radiata*

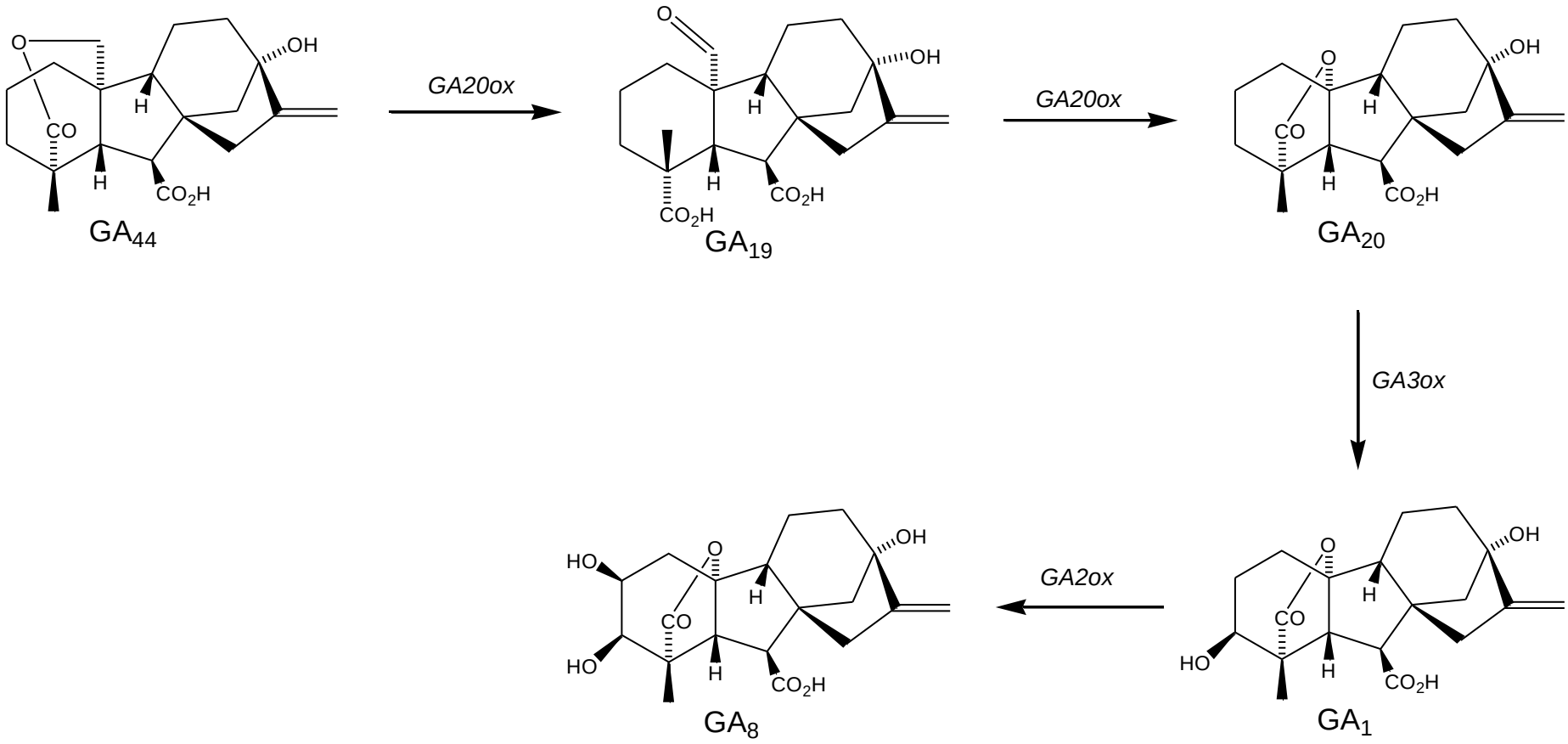
A Fast Growing Hybrid Cross in NZ

A Initial Working Hypothesis: Inherently Fast-growing Poplar Trees (Families & Individual Genotypes) Have Higher Levels of 'Growth-effector' Gibberellin (GA_1) in Growing Wood (Xylem) & Bark Tissues & Higher Levels of Both GA_1 and GA_4 in Elongating Internode Tissues

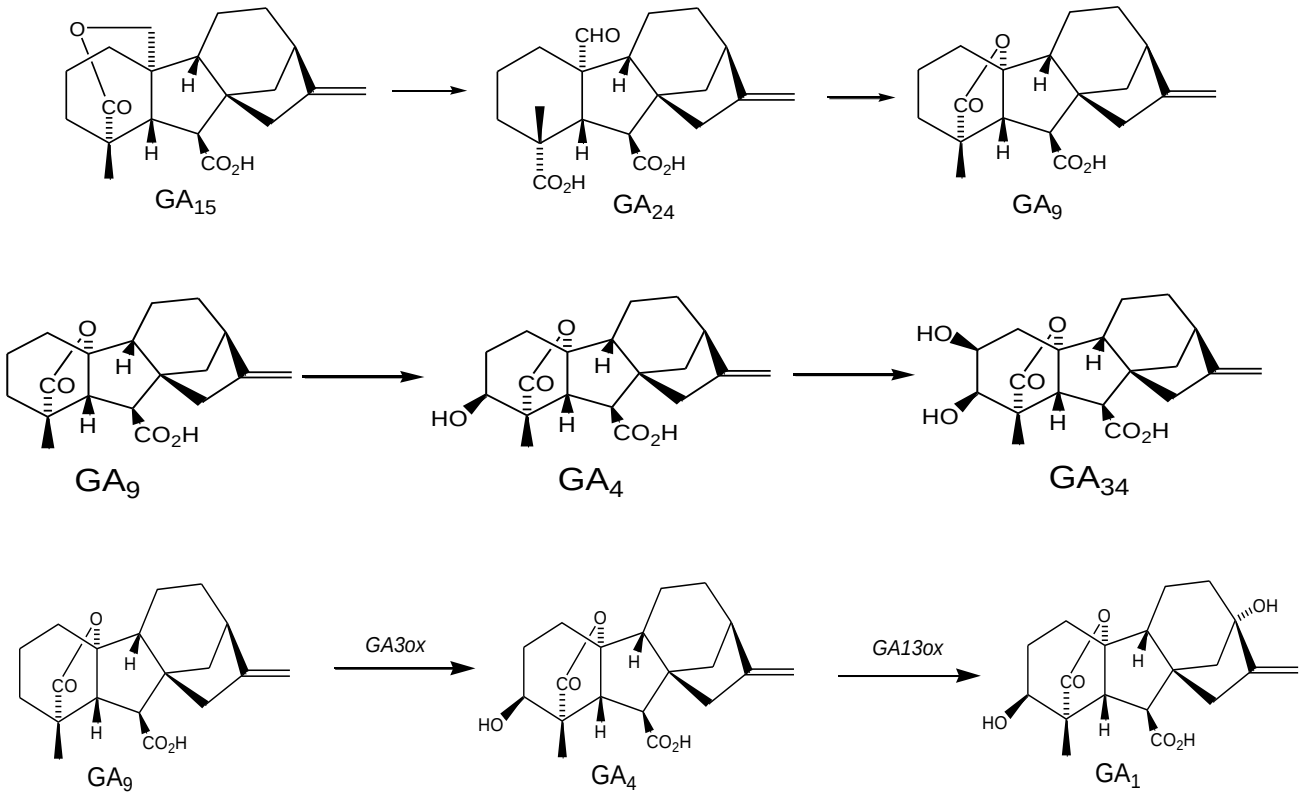
Gibberellin A_1



One Biosynthesis Pathway for Synthesis of the Growth Active Gibberellin, GA_1



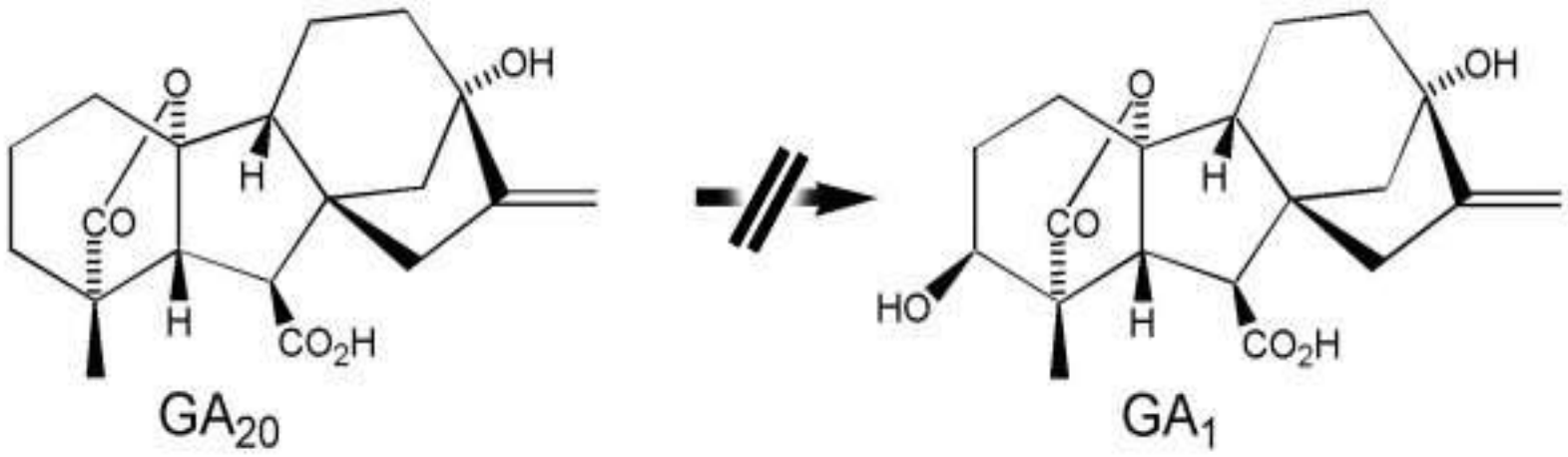
A 2nd Biosynthesis Pathway for Synthesis of the Growth Active Gibberellin, GA_4 (and Possibly GA_1 Also)

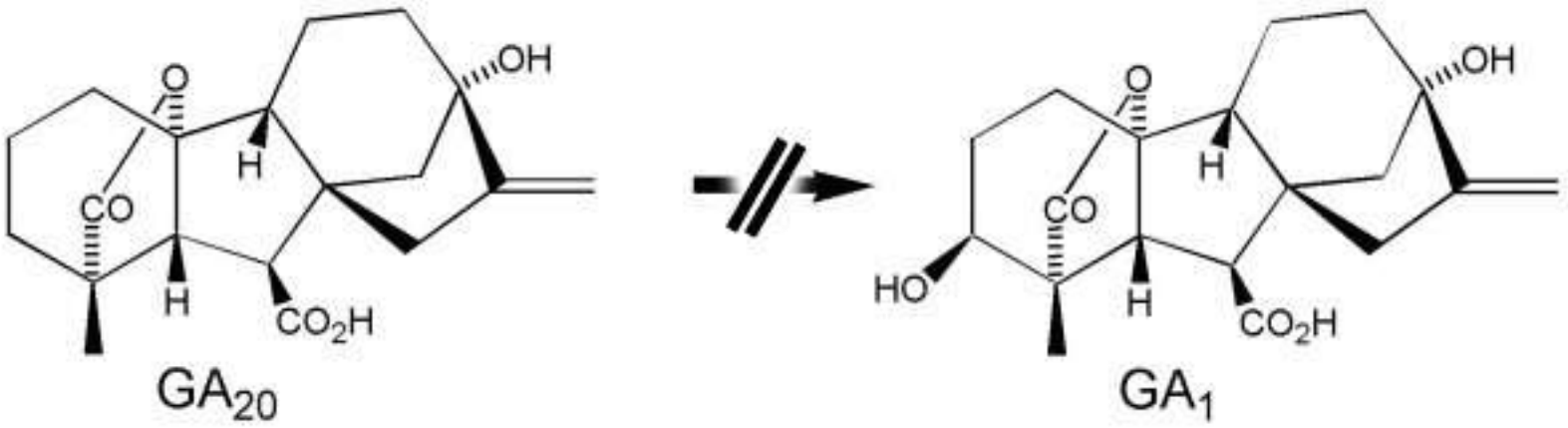


Another Hypothesis: Gibberellin A_1 is Required Not Only for Radial & Height Growth of the Tree Trunk, But Also for Xylem Fibre Length.

3β-hydroxylation of GA_{20} to GA_1 was blocked in *Eucalyptus globulus* by use of the acylcyclohexanedione, trinexapac ethyl (Syngenta)

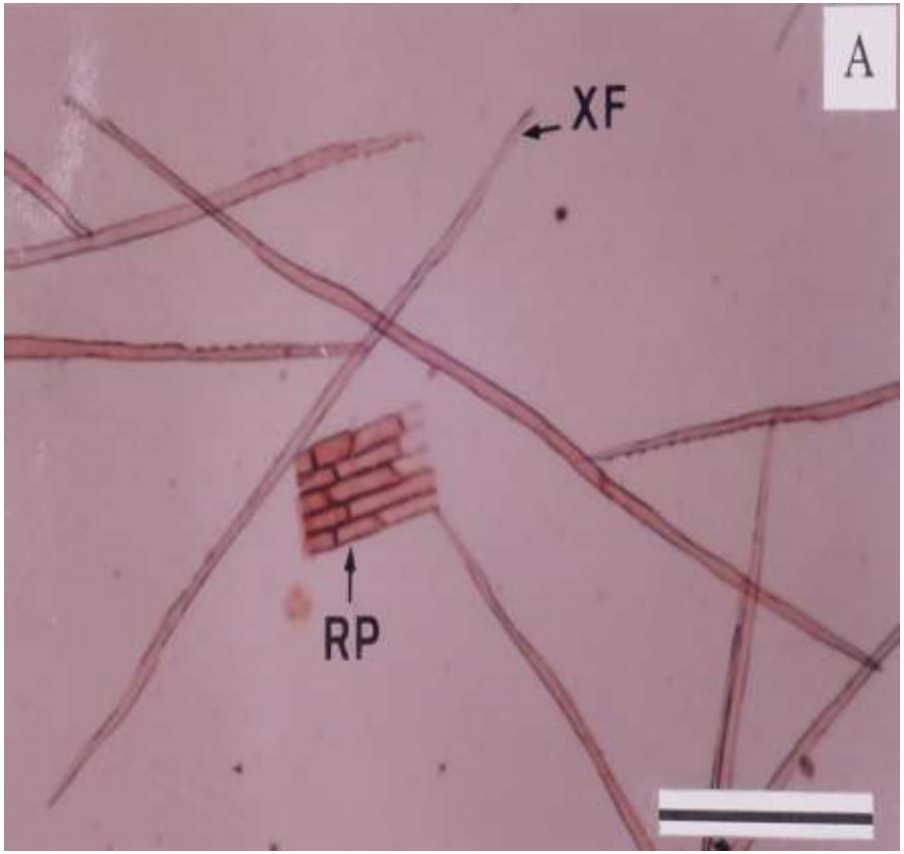
-- Ridoutt, B.G., R.P. Pharis & R. Sands. 1996. Secondary xylem fibre development ……. Physiol. Plant. 96:559-566.



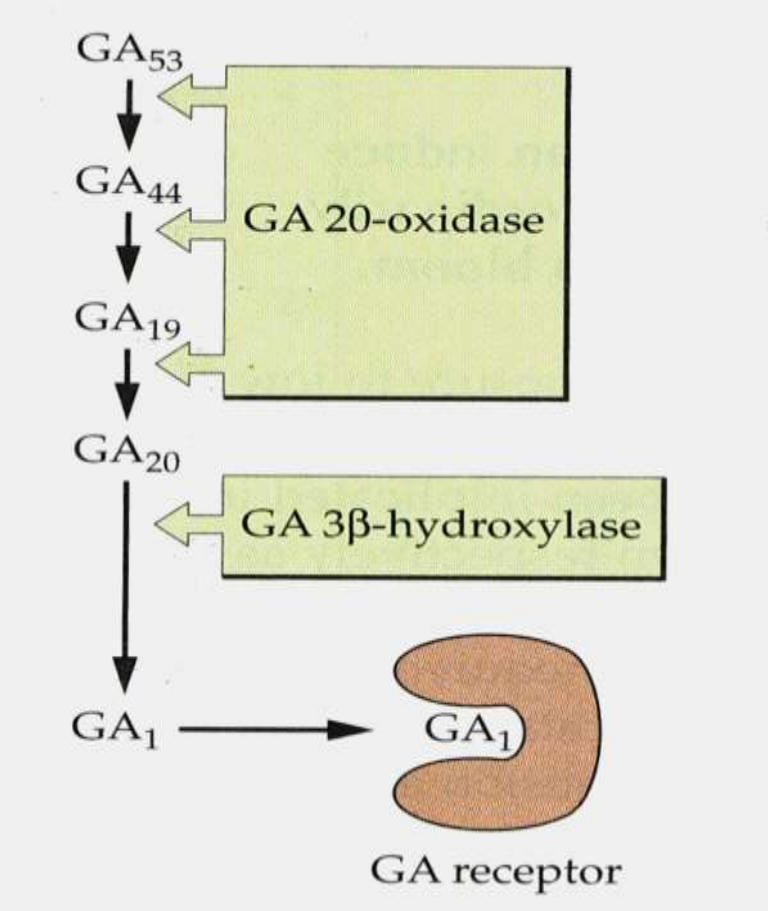


Control (XF=xylem fibre)

GA_1 synthesis blocked



Overexpression of the *GA20ox* Gene in Hybrid Aspen Gives Increased Gibberellin Biosynthesis, Increased Radial Stem Growth & Longer Xylem Fibres



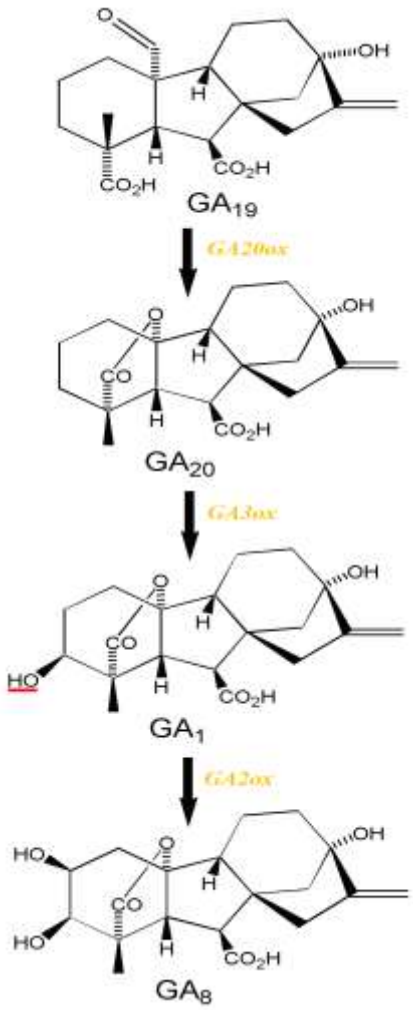
Control Xylem Fibres

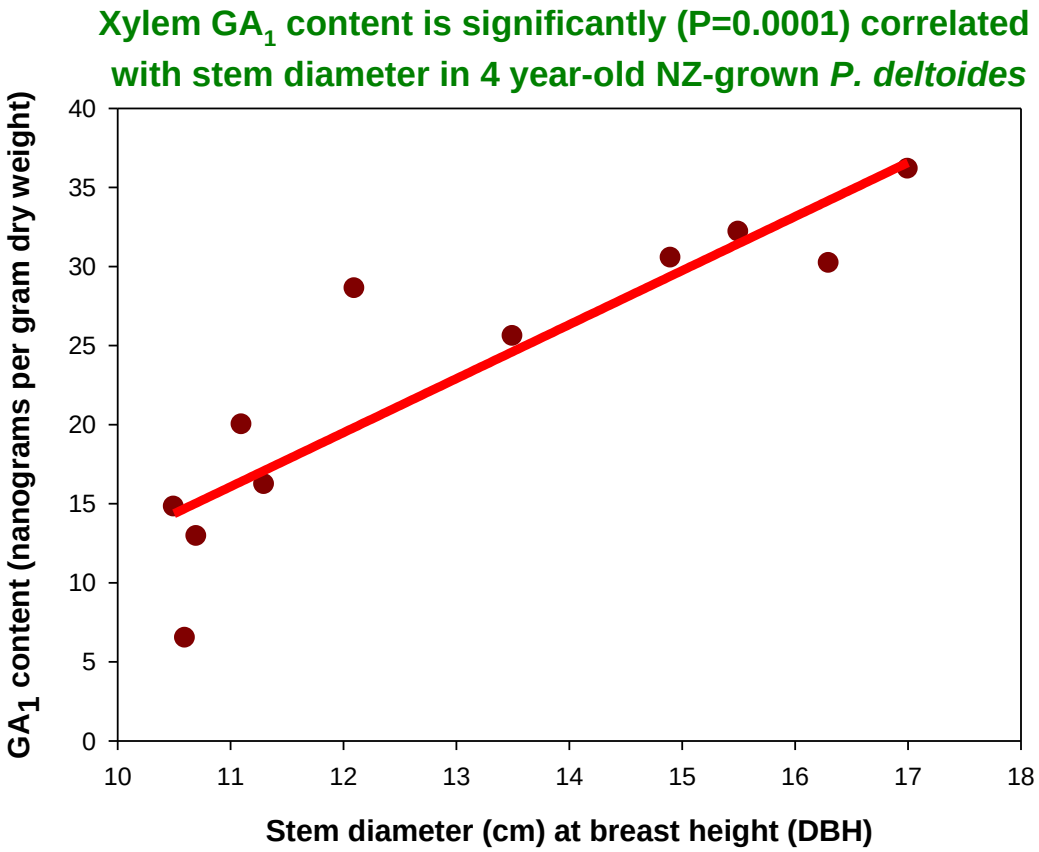
GA20ox Overexpressed Xylem Fibres

Eriksson et al., 2000

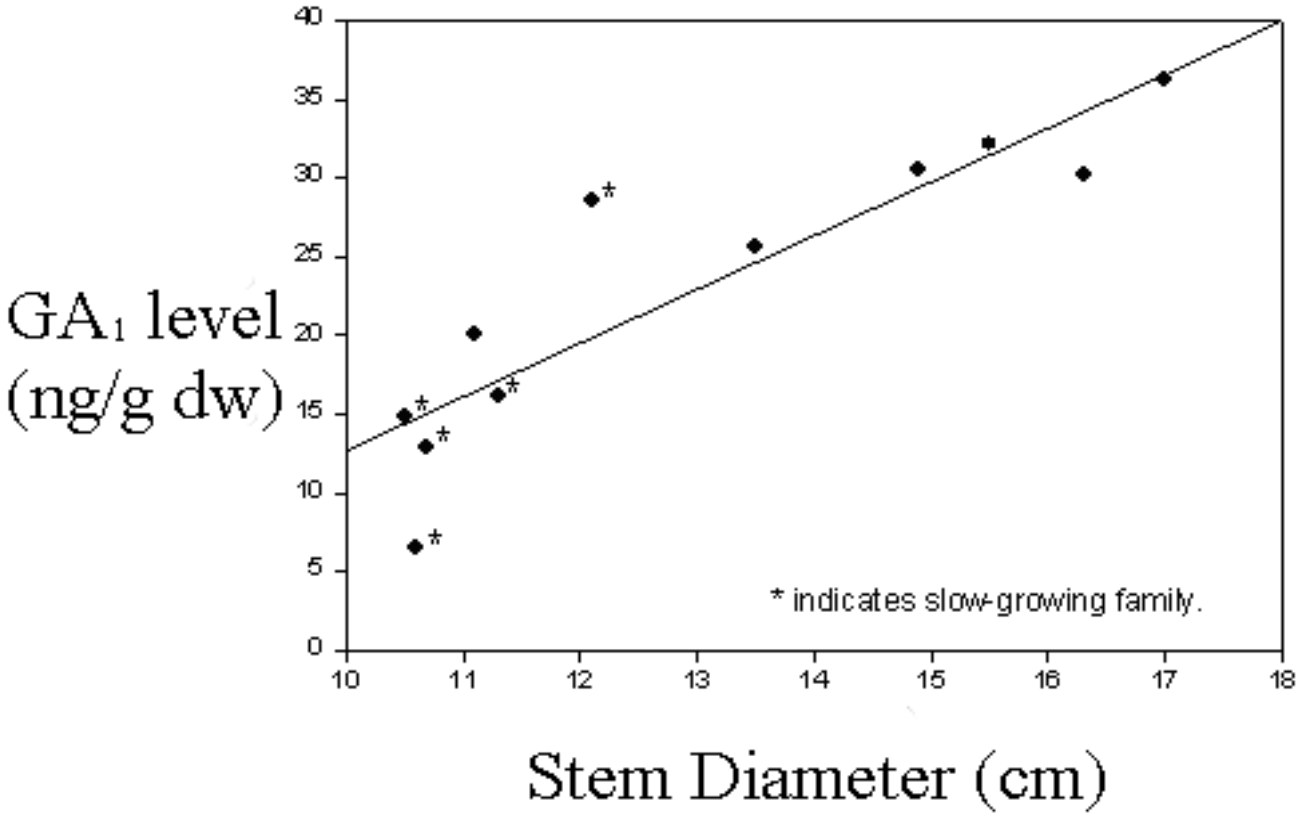
Question: Are Plant Hormones Causal For Radial Stem Growth In Poplar?

Gibberellin A1 - a proven effector of shoot elongation.





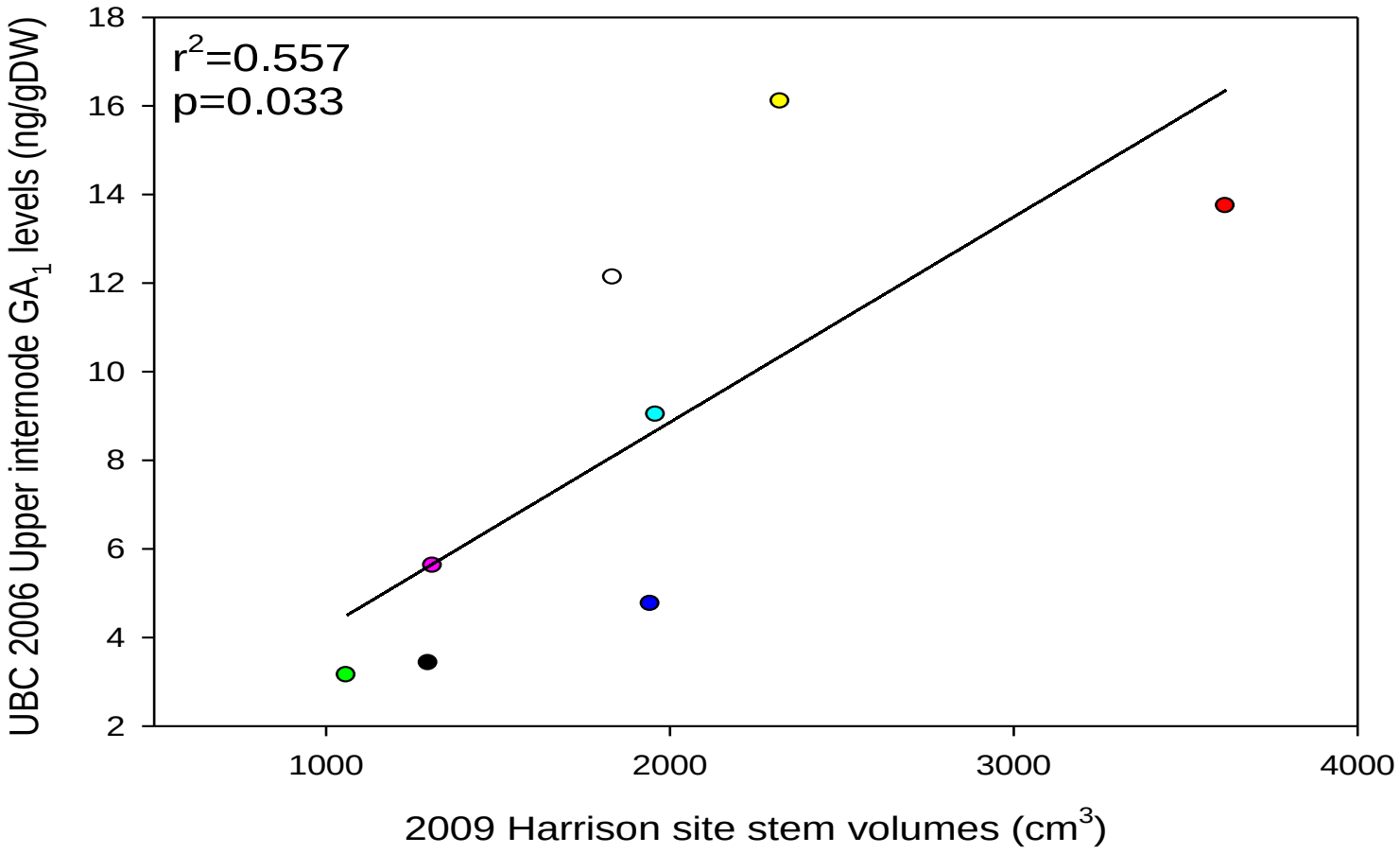
GA_1 Content in Growing Xylem Tissue "Reflects" (is Significantly (P=0.001) Correlated with) Year 4 Stem Diameters **Across Genotype** for 4 year-old NZ-grown *P. deltoides*



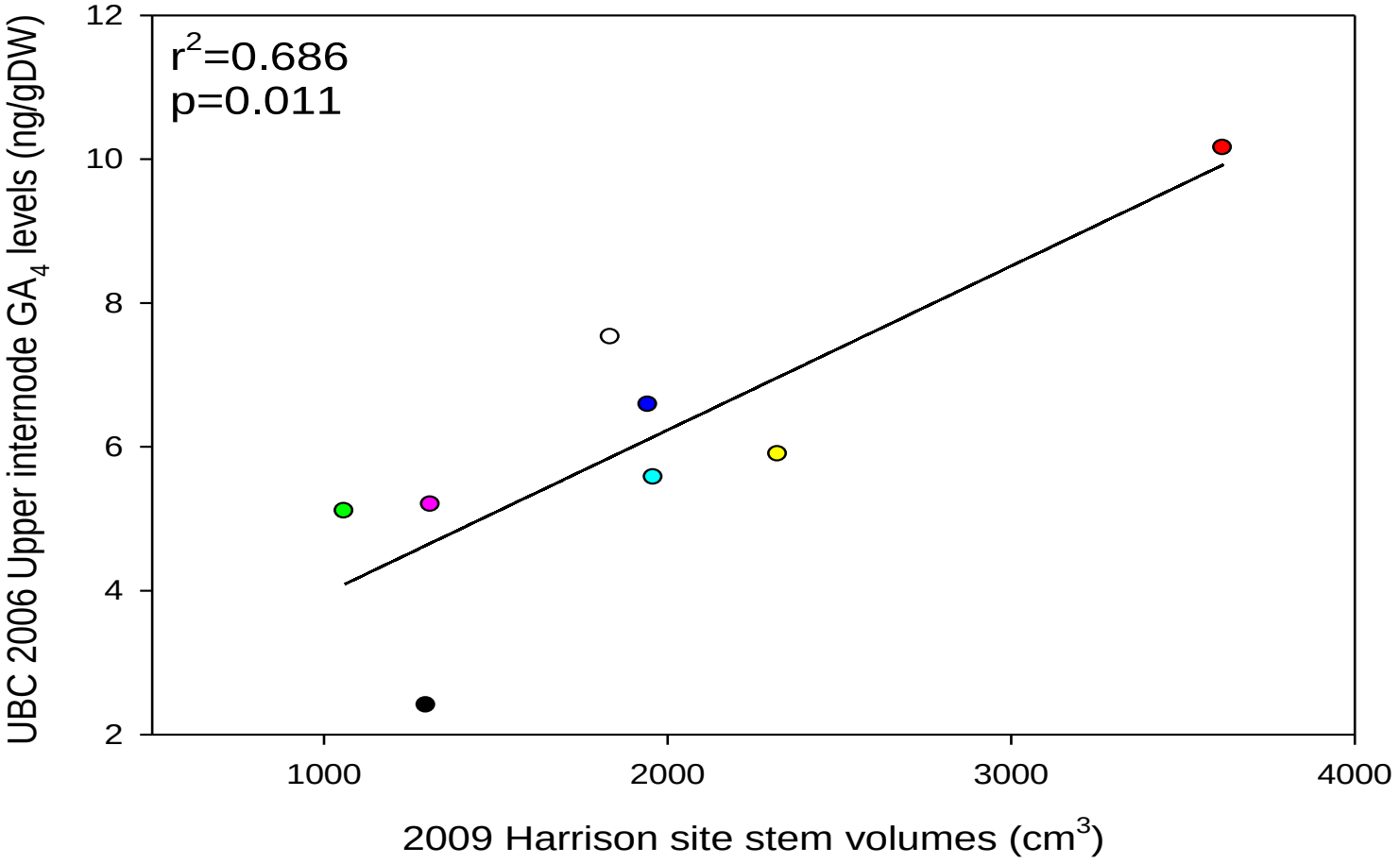
Answer: **"Yes", GA_1 is a highly significant variable for stem diameter growth across genotype (n = 11) and the slow-growing family (Oklahoma parents) has a significantly (P = 0.01) lower concentration of GA_1 in growing xylem tissues than the faster-growing family (Mississippi parents)**

Question: What about other poplar species which are also adapted to ‘temperate climates’, such as *P. trichocarpa*? When “across genotype” is set as the experimental variable, do the same or similar significant correlations with plant hormone concentrations also hold true?

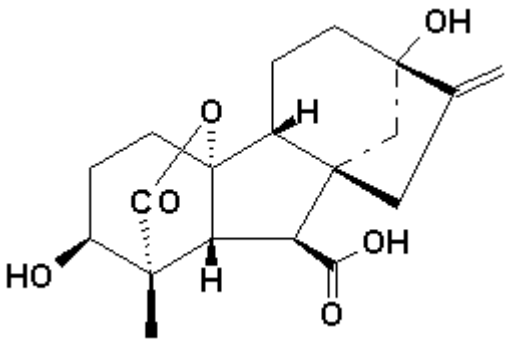
June 2006 UBC Vertical Whips [Coppiced]. **Upper Internode** Tissue GA_1 Levels at Age 90 days were Correlated with Tree Stem Volumes at Age 3 Years (October 2009) in a Field Provenance Trial Near Harrison Hot Springs for the Same 8 Genotypes



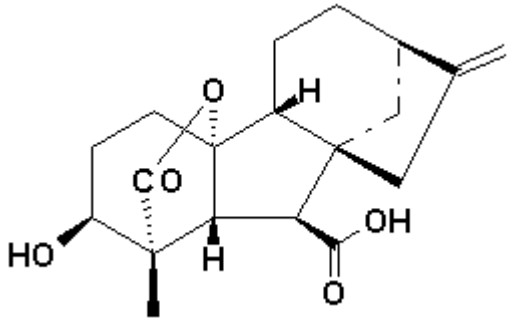
June 2006 UBC Vertical Whips [Coppiced]. **Upper Internode** Tissue GA_4 Levels at Age 90 days were Correlated with Tree Stem Volumes at Age 3 Years (October 2009) in a Field Provenance Trial Near Harrison Hot Springs for the Same 8 Genotypes



Structures of the Two Growth 'Effector' Gibberellins Identified and Quantified in Rapidly Elongating Internode Tissues of *Populus trichocarpa* Coppiced "Whips" that were Harvested in Mid-June 2006

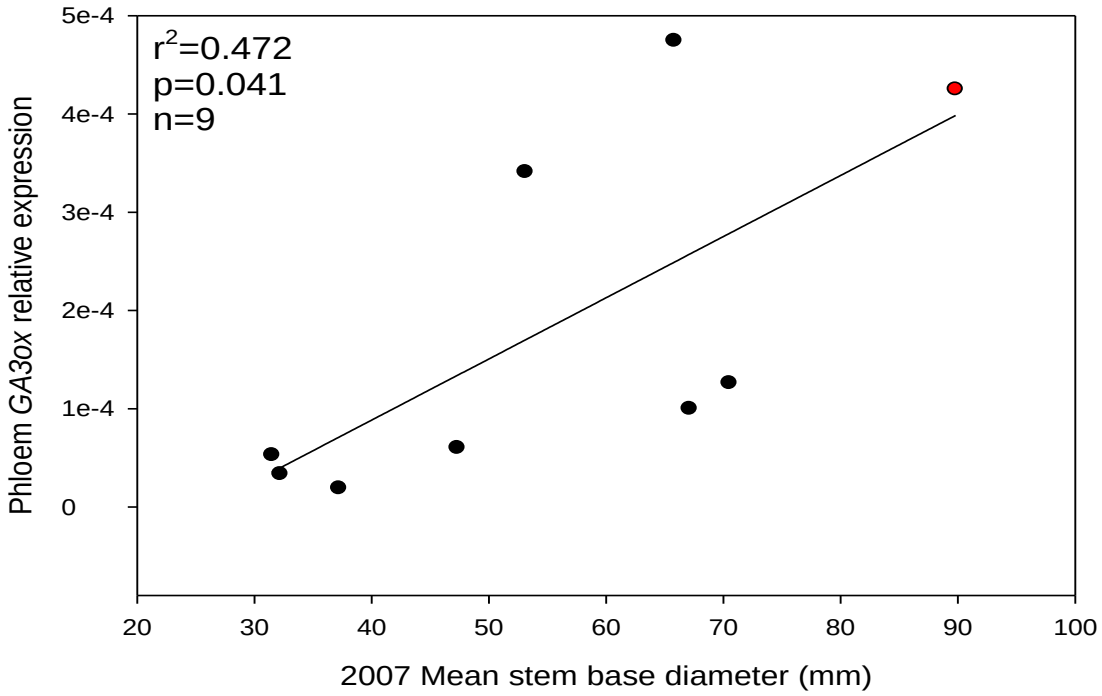


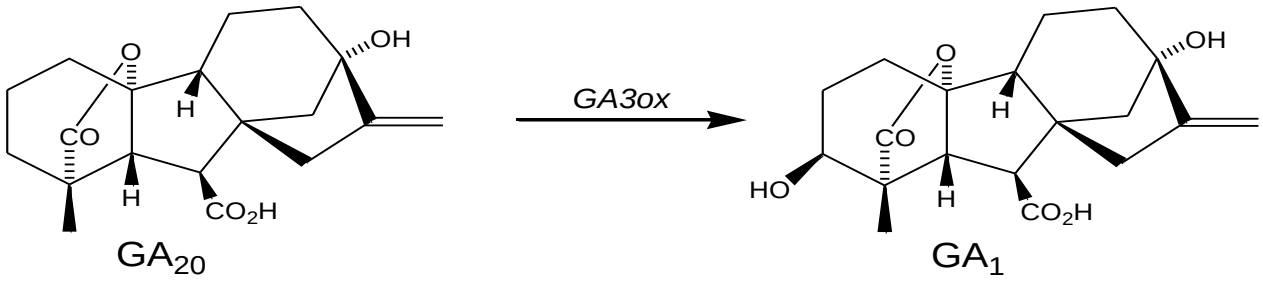
Gibberellin A_1



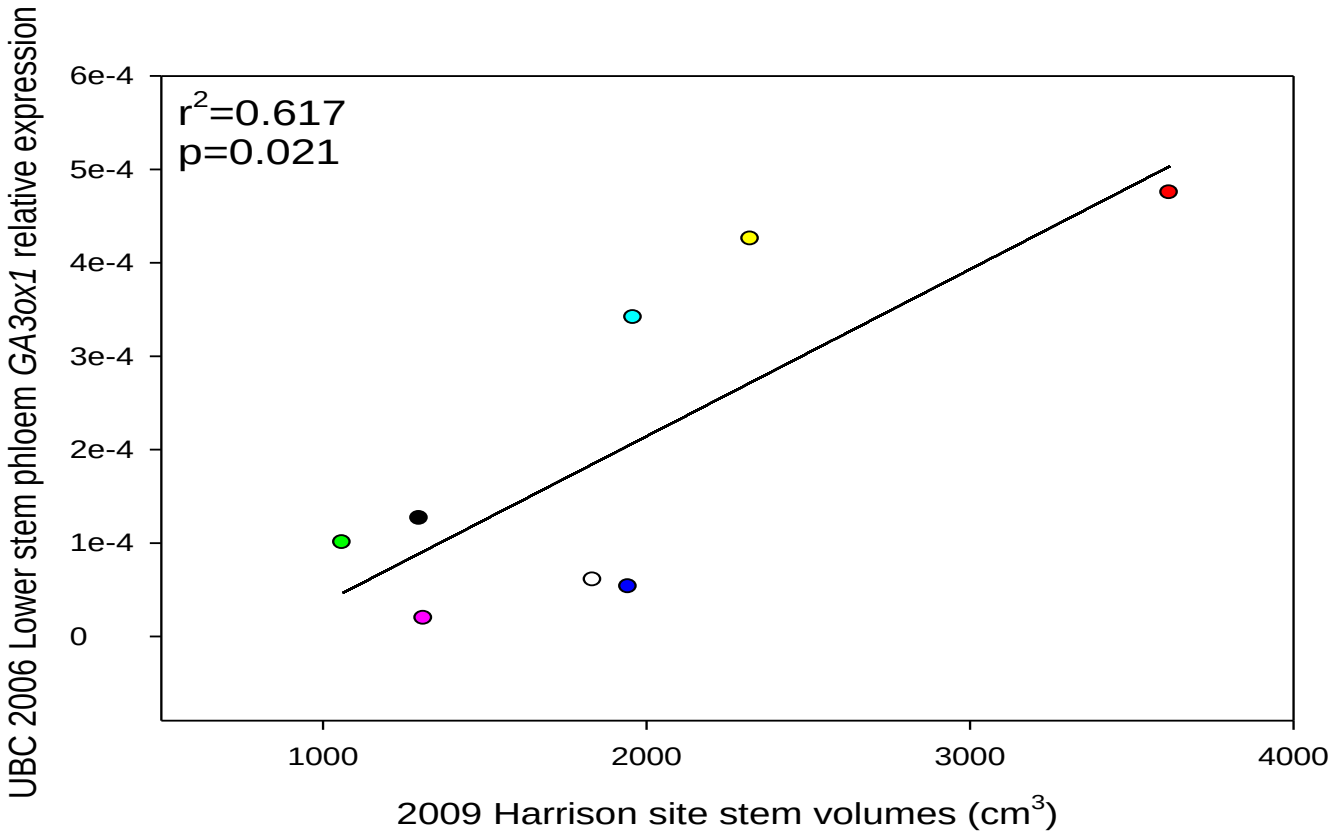
Gibberellin A_4

A comparison of relative levels of gene expression of *GA3ox1* (June 2006 vertical whips produced by coppice cutting – tissue was scraped from the lower stem phloem side) with July 2007 stem basal diameters of the same 9 *Populus trichocarpa* genotypes -- Totem Field, UBC

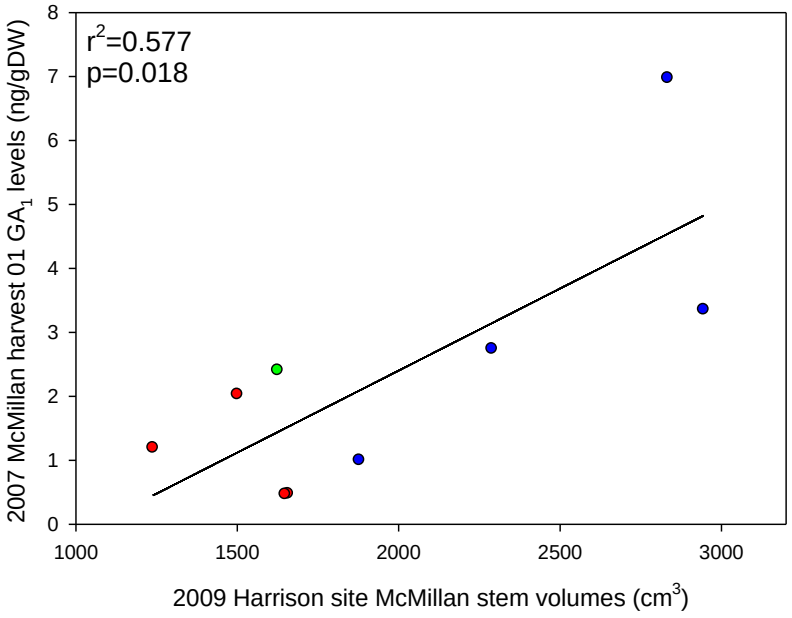


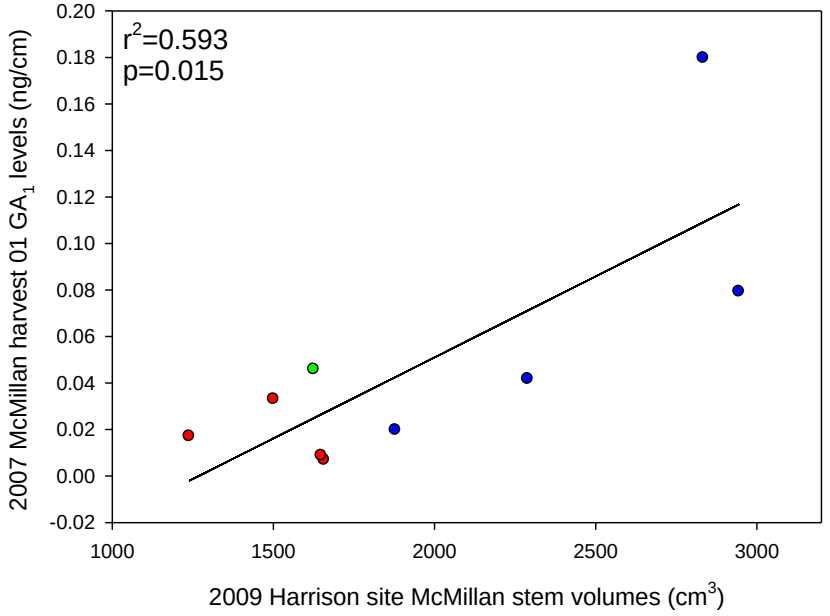


Relative levels of gene expression of *GA3ox1* in mid-June 2006. The vertical whips had been produced by coppice cutting. Tissue for analysis was scraped from the phloem side of the lower stems of the whips at age 3 months. *GA3ox1* expression was then correlated with tree stem volumes [age 3 years in 2009] across genotype in a Field Provenance Trial near Harrison Hot Springs.

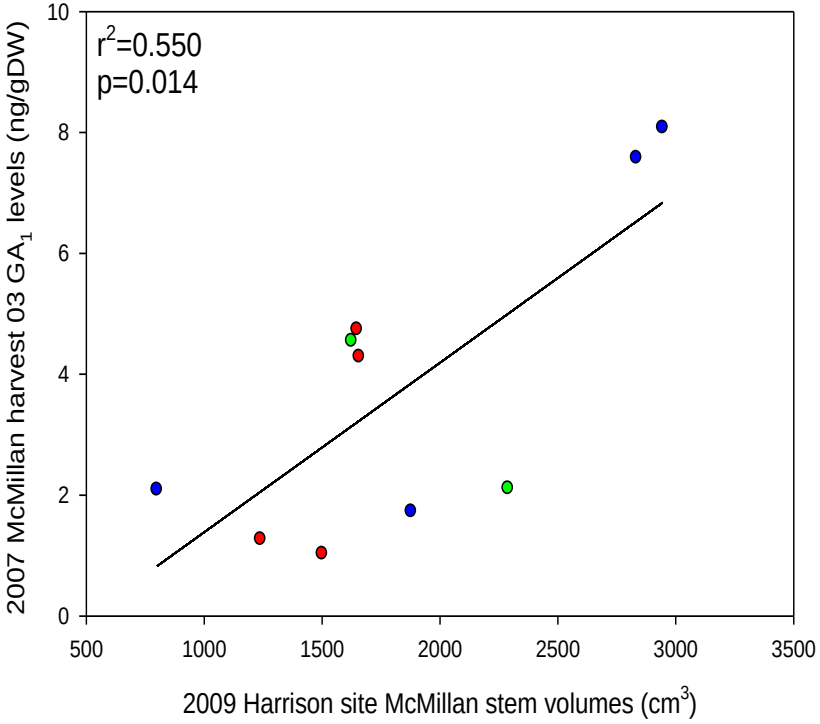


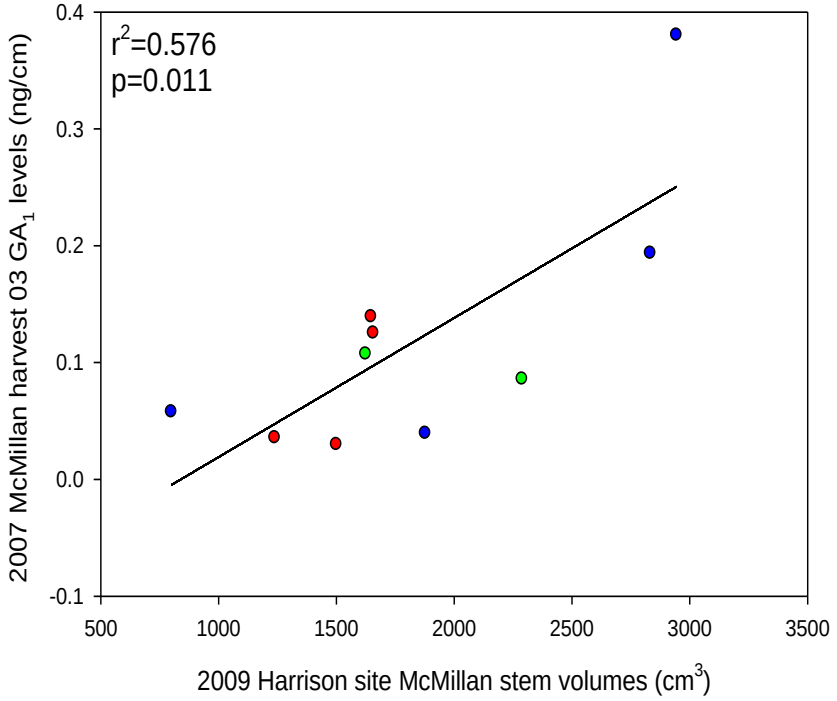
Nine randomly selected genotypes from the McMillan Islands provenance where elongating internode tissue was analyzed for GA_1 at age 30 days and related to (correlated with) stem volumes of the same genotypes in a provenance field growth trial near Harrison Hot Springs at age 3 years



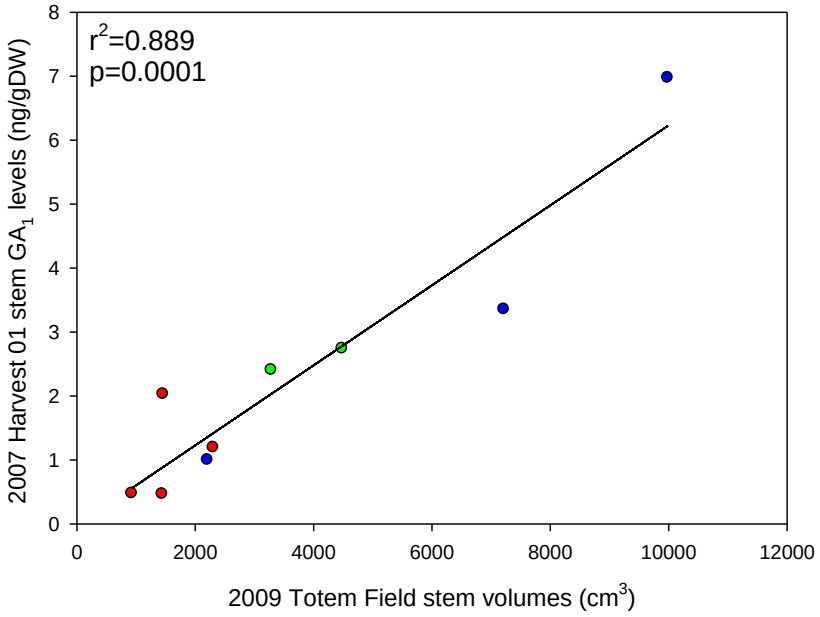


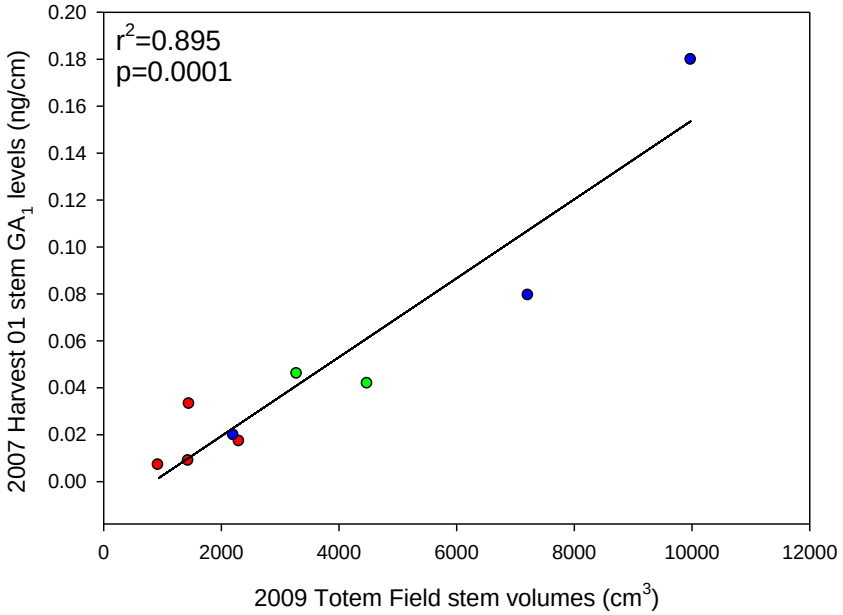
Ten randomly selected genotypes from the McMillan Islands provenance. Elongating internode tissue was analyzed for GA_1 at age 90 days and related to stem volumes of the same genotypes in a provenance field growth trial near Harrison Hot Springs at age 3 years



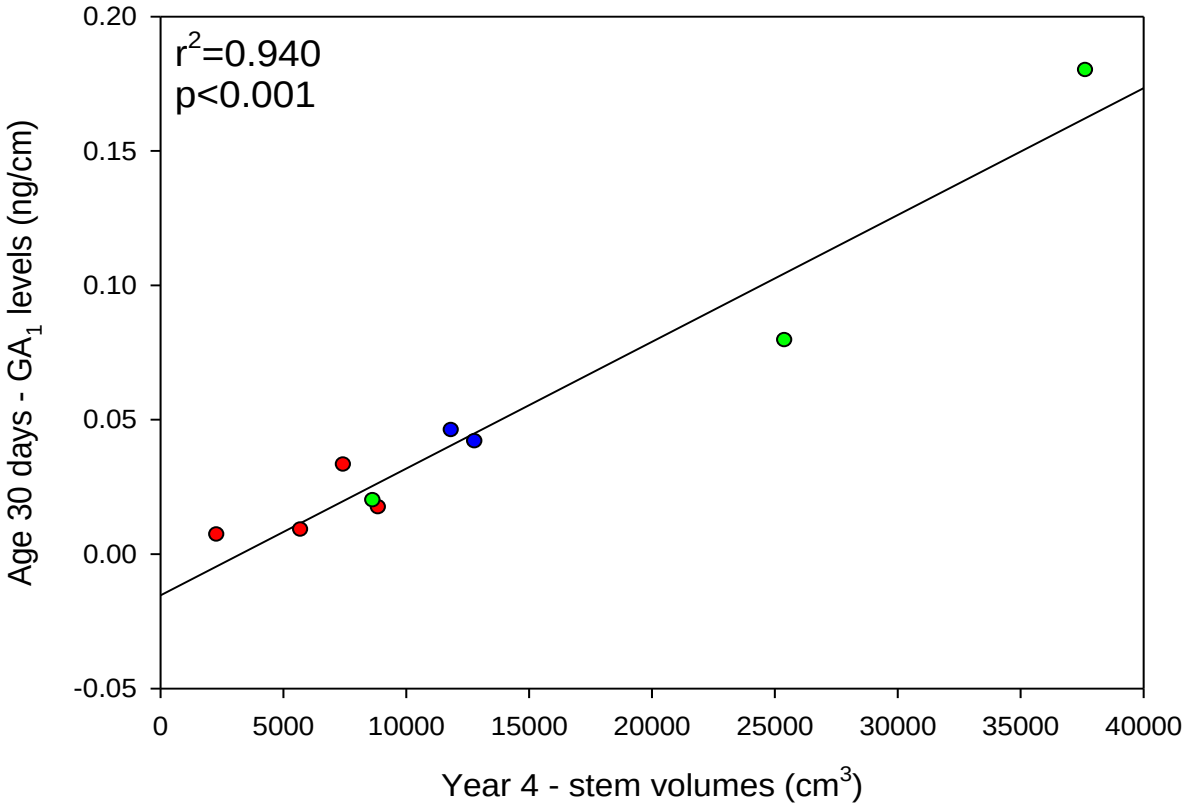


Nine randomly selected genotypes from the McMillan Island, B.C. provenance. Elongating internode GA1 concentrations were measured at age 30 days and correlated **across genotype** with stem volumes for the same genotypes in UBC's Totem Field growth trial [which was irrigated] in January 2011 after 3 summers of growth





Nine randomly selected genotypes from the McMillan Island, B.C. provenance. Elongating internode GA_1 concentrations were measured at age 30 days and correlated **across genotype** with stem volumes for the same genotypes in UBC's Totem Field growth trial [which was irrigated] in January 2011 after 4 summers of growth

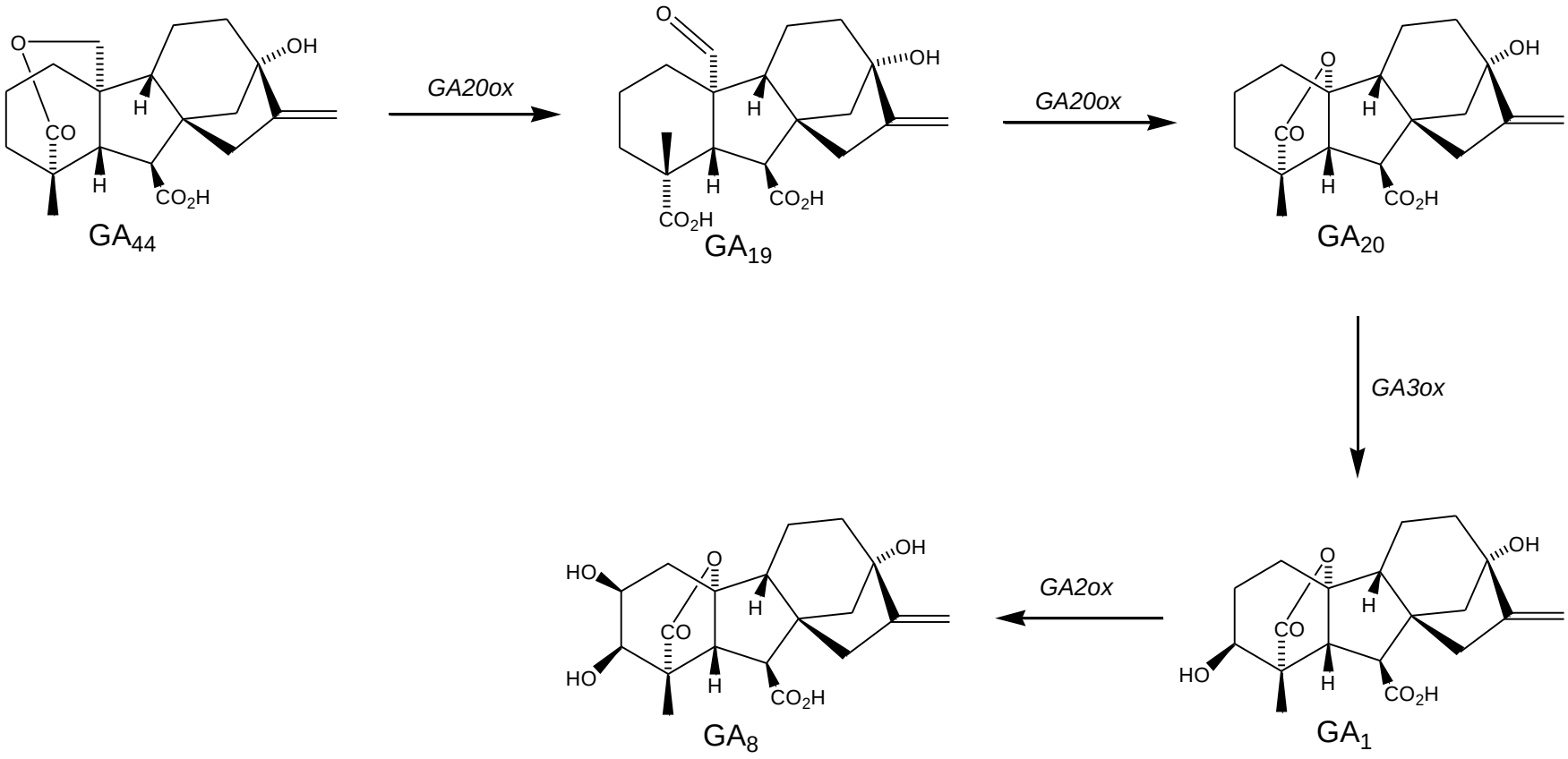


CONCLUSIONS: For two poplar species, *P. deltoides* and *P. trichocarpa*, and for their **genotypes that are "adapted" to temperate climates,** the concentrations of "growth effector" gibberellins (GA1 and/or GA4) growing xylem and internode tissues reflects inherent stem growth capacity across a wide range of genotypes.

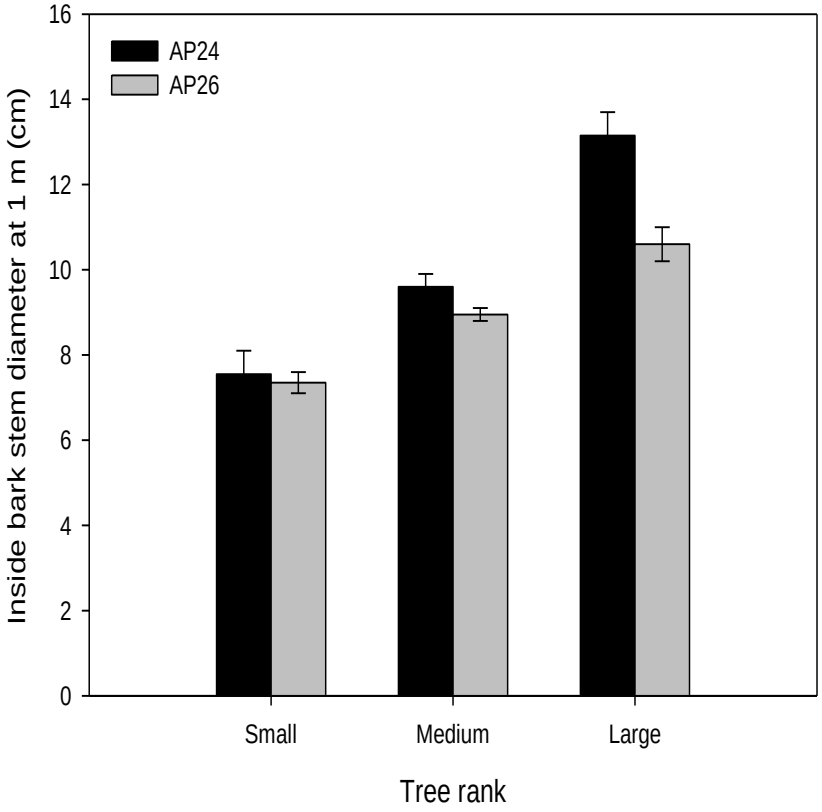
That "reflection" may be useful as a "predictive marker" in choosing inherently fast-growing genotypes for use in afforestation and agroforestry.

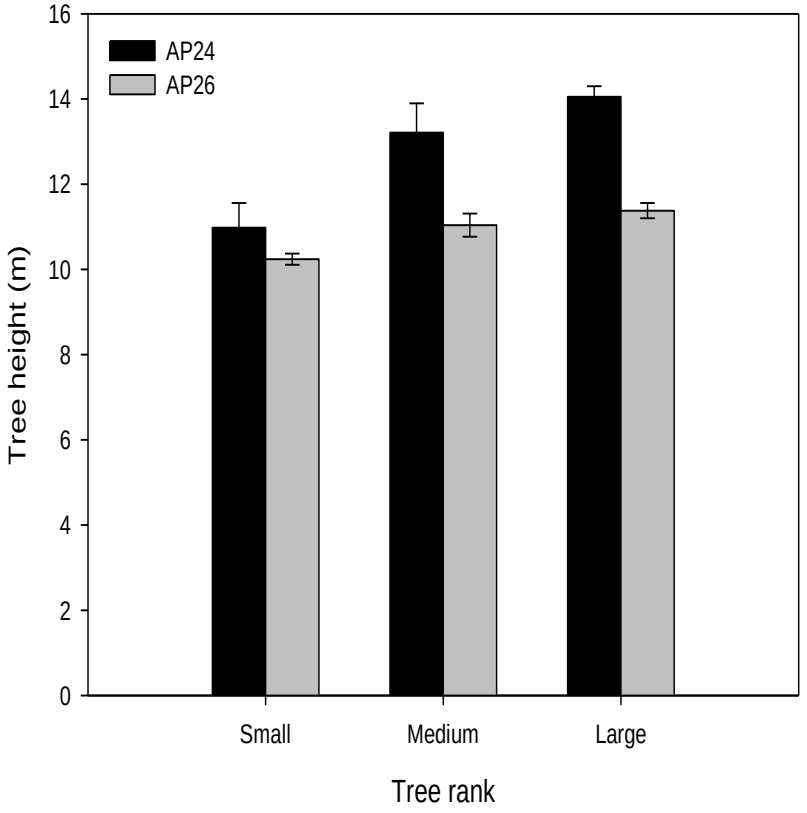
Further, for *P. trichocarpa*, at least, it appears that the GA biosynthesis "activation step" - 3β-hydroxylation, catalyzed by GA3 oxidase and encoded for by *GA3ox1* , is a rate-limiting step across genotype for selections from the natural gene pool.

Question: Are gibberellin & other growth hormone concentrations also significantly correlated with stem growth of balsam poplar and hybrid poplar genotypes which are adapted to Canada's "interior" provinces, with their "short summers, very long days & early autumn Arctic cold fronts ?

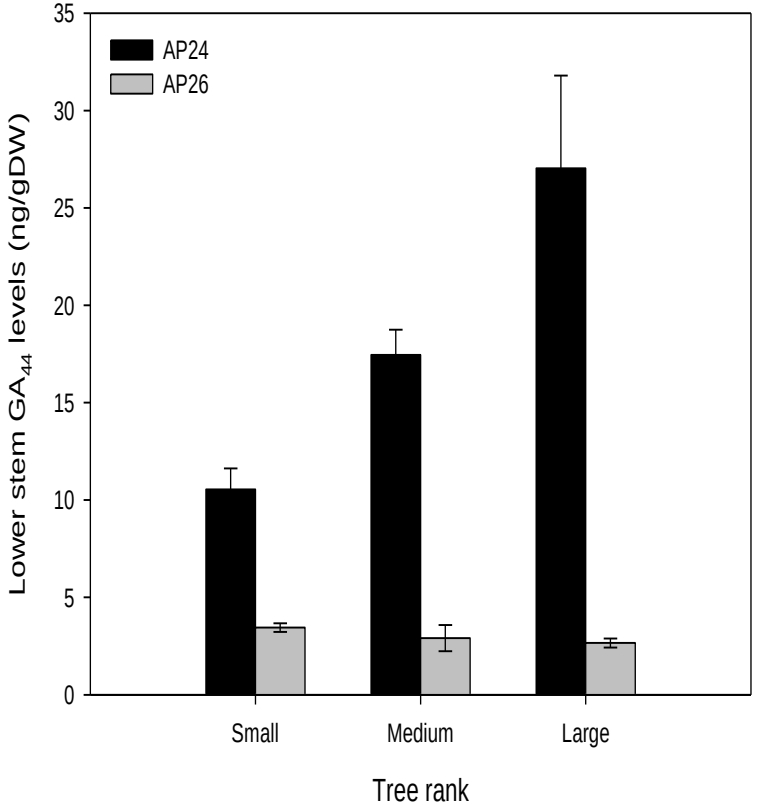


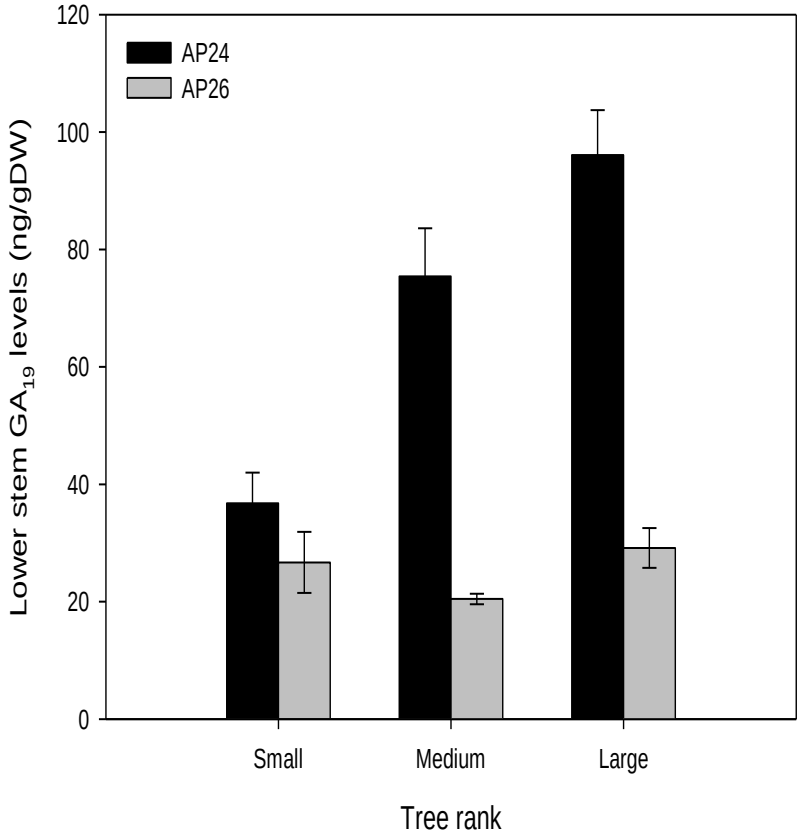
Two “N. Alberta-adapted” Hybrid Poplars, cv. ‘Walker’ (AP24) and cv. ‘Manitou’ (AP26). Two trees were chosen to represent stem diameter “rank” within the randomized provenance trial, for each clone. Stem Diameters and Heights at ca. 12 Years of Age



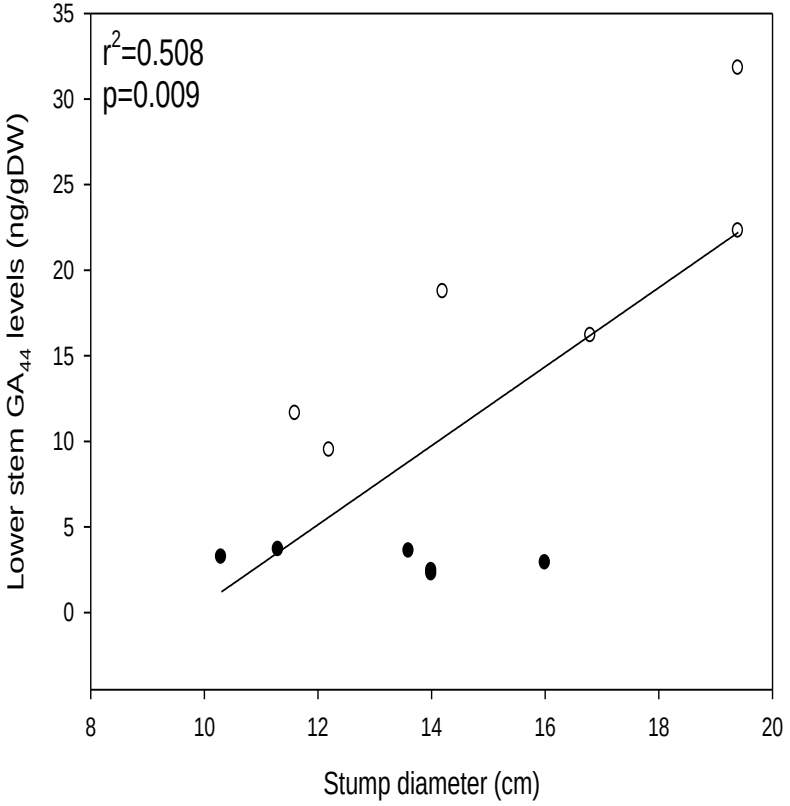


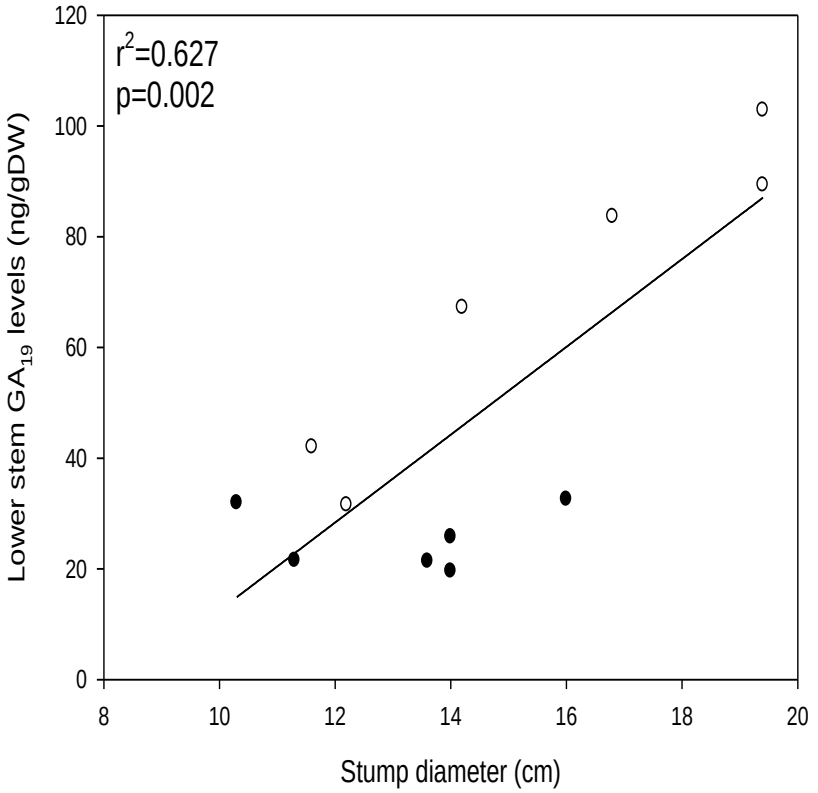
Two “N. Alberta-adapted” Hybrid Poplars, cv. ‘Walker’ (AP24) and cv. ‘Manitou’ (AP 26). Comparisons of Growth Hormone Correlations with Stem Diameter “Rank”. Each ‘bar’ represents the mean GA levels for 2 ramets. The GA_{44} → GA_{19} Biosynthetic Step is catalyzed by GA20 oxidases & encoded for by *GA20ox*



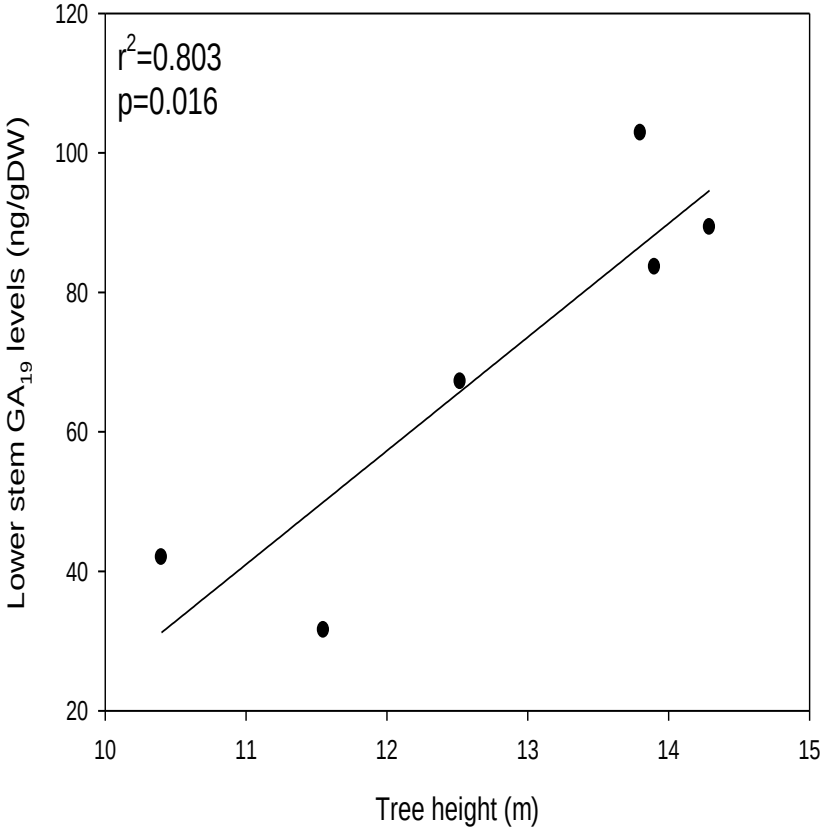


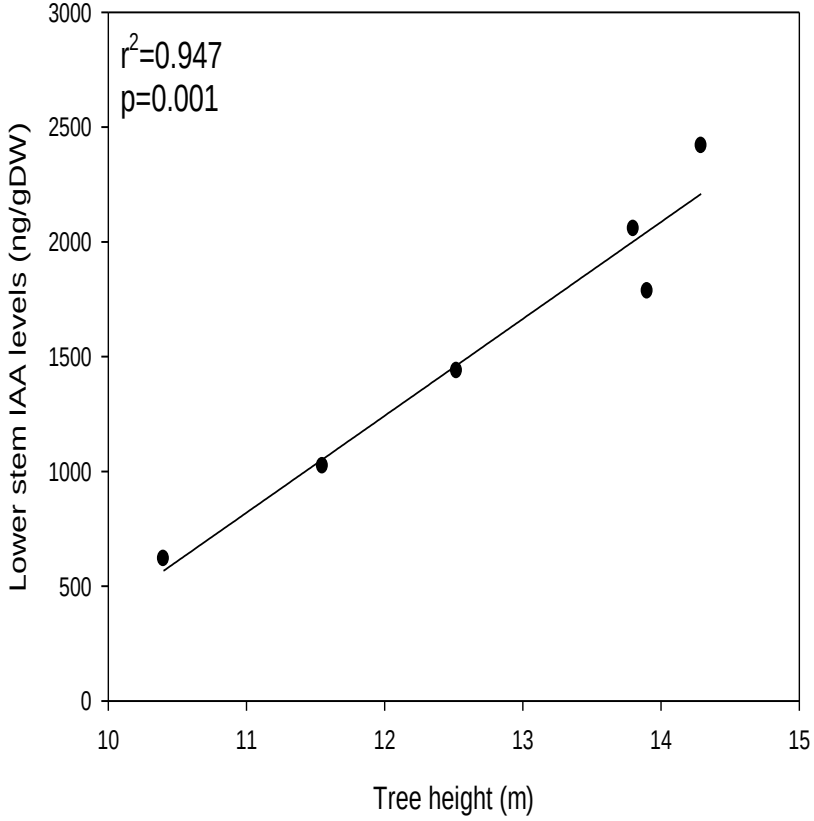
Two “N. Alberta-adapted” Hybrid Poplars, cv. ‘Walker’ (6 ramets with open circles) and cv. ‘Manitou’ (6 ramets with closed circles). Comparisons of Growth Hormone (GA_{44}, left & GA_{19}, right) with Stump Diameters. The GA_{44} → GA_{19} Biosynthetic Step is catalyzed by GA20 oxidases & encoded for by *GA20ox*



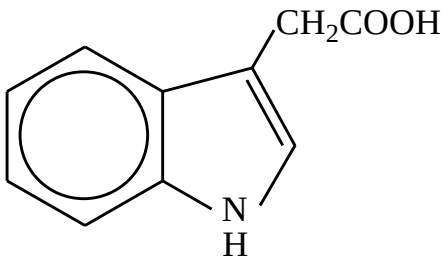


An Alberta-adapted" Hybrid Poplar, cv. 'Walker' (6 ramets with closed circles). Comparisons of Growth Hormone Concentration (GA_{19}, left & the auxin, IAA, right) with Tree Heights **Across Ramet within Clone.** The $GA_{44} \rightarrow GA_{19}$ Biosynthetic Step is encoded for by *GA20ox*. IAA is known to regulate GA metabolism (reviewed in Thomas & Hedden, 2006, page 154).

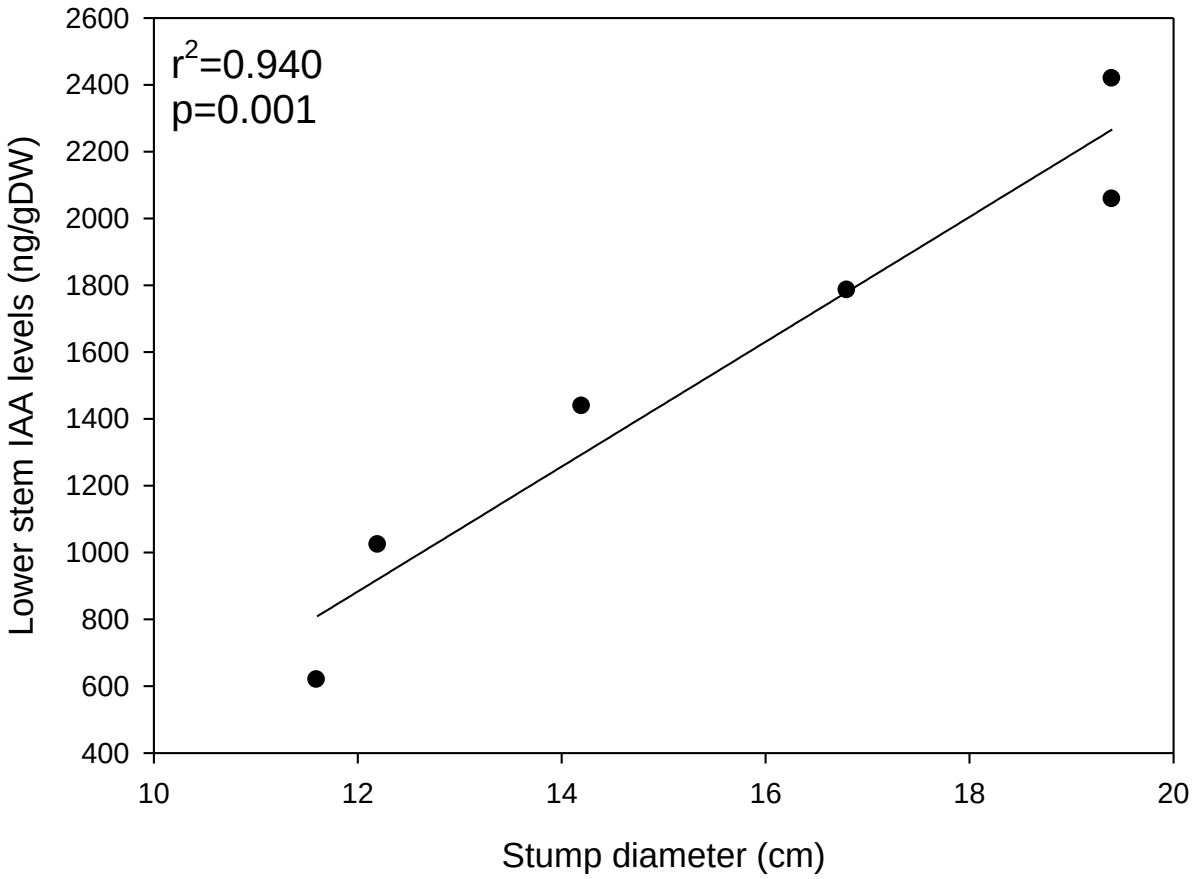




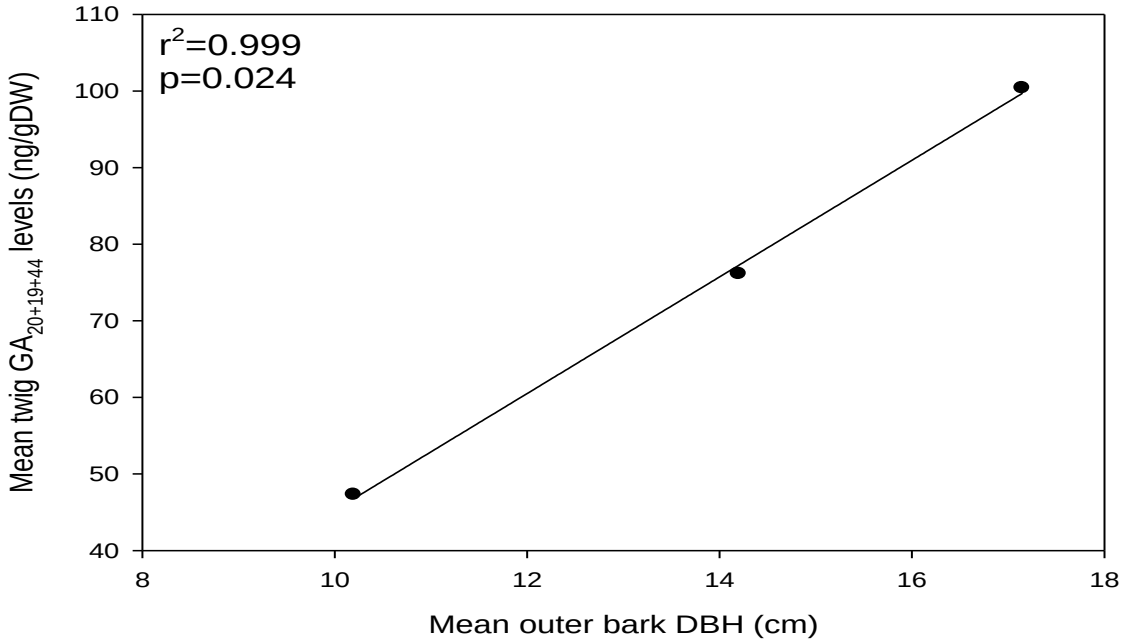
Within Genotype Variation in Stump Diameters Across Ramet – The relationship of indole-3-acetic acid (IAA) concentrations in lower stem xylem tissues (harvested in mid-July 2005) to stem diameters **across 6 ramets** of Al-Pac hybrid poplar cv. Walker trees. A similar correlation occurs for GA19 (data not shown).

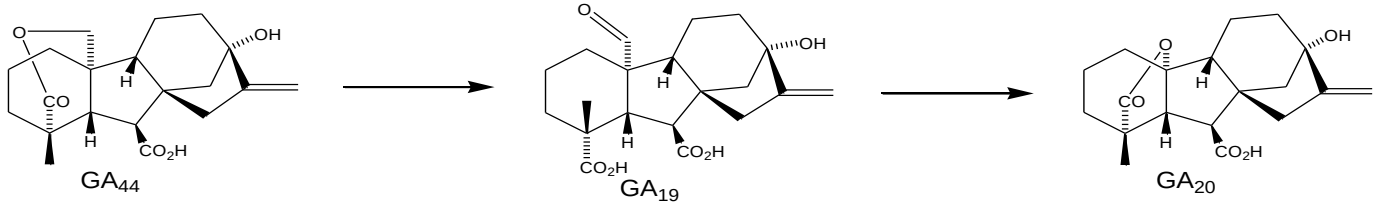


IAA



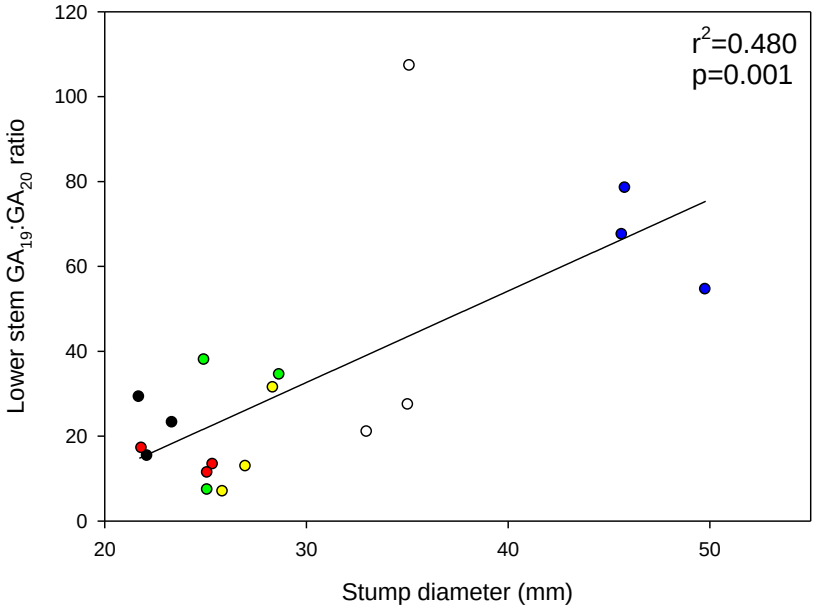
Within Genotype Variation in Stem Diameters (DBH) Across Ramet – The sum of GA44 + GA19 + GA20 concentrations in upper "twig" tissues ('elongating internodes' harvested in mid-July), when correlated across ramet, "**reflects**" tree DBH for cv. Walker, an Alberta-adapted hybrid poplar. Each closed circle is the mean of two ramets for both DBH & the GA concentration.

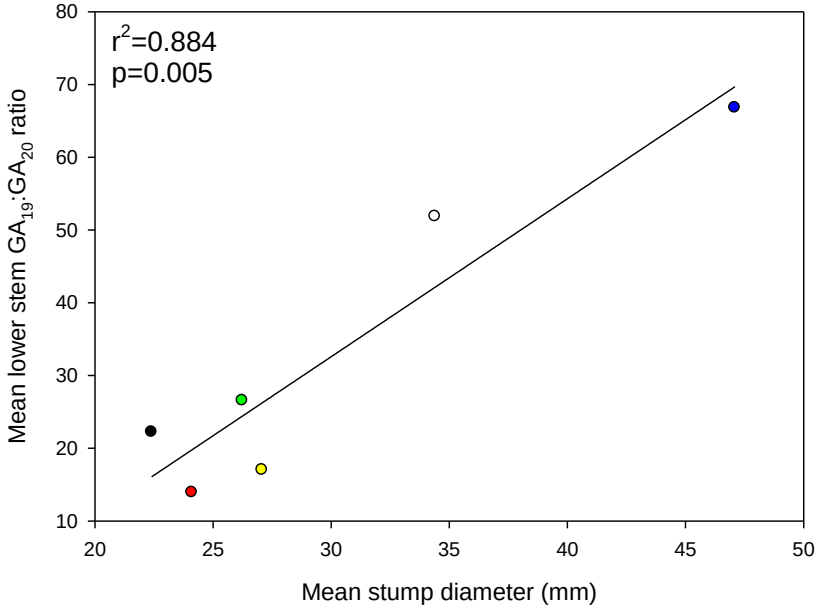


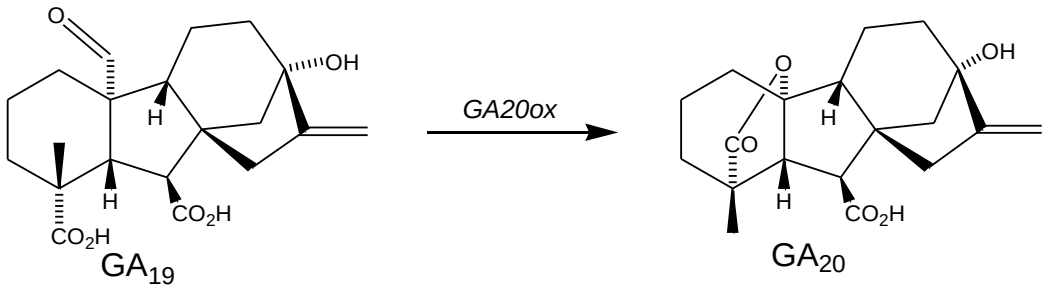


Indian Head SK 2006 Hybrids: 6 genotypes X 3 ramets. Xylem tissue was harvested in early August for hormone analysis – The ratio of ‘precursor’ GA19 to ‘product’ GA20 was correlated with tree stump diameter

WP-69 →Tristis (white) → **Walker** → **Hill (yellow)** → **CanAm** →**Max (black)**

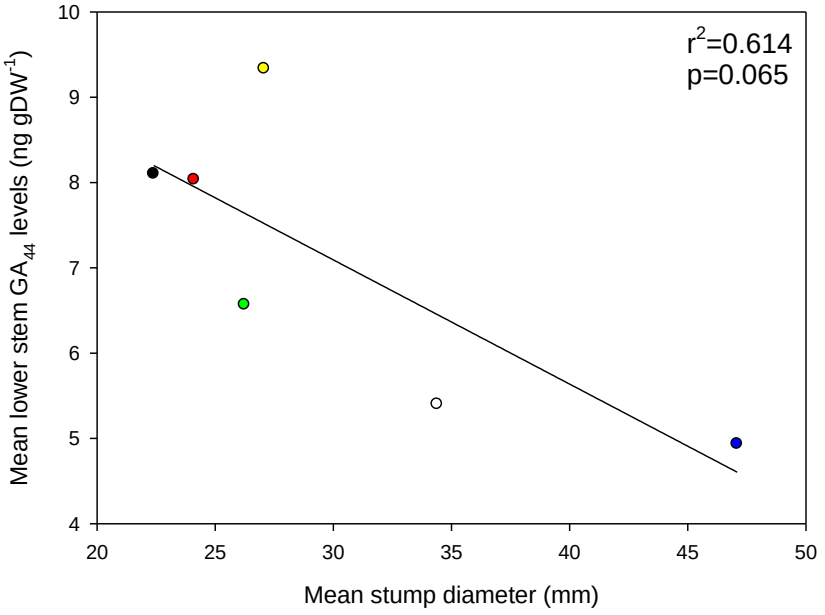


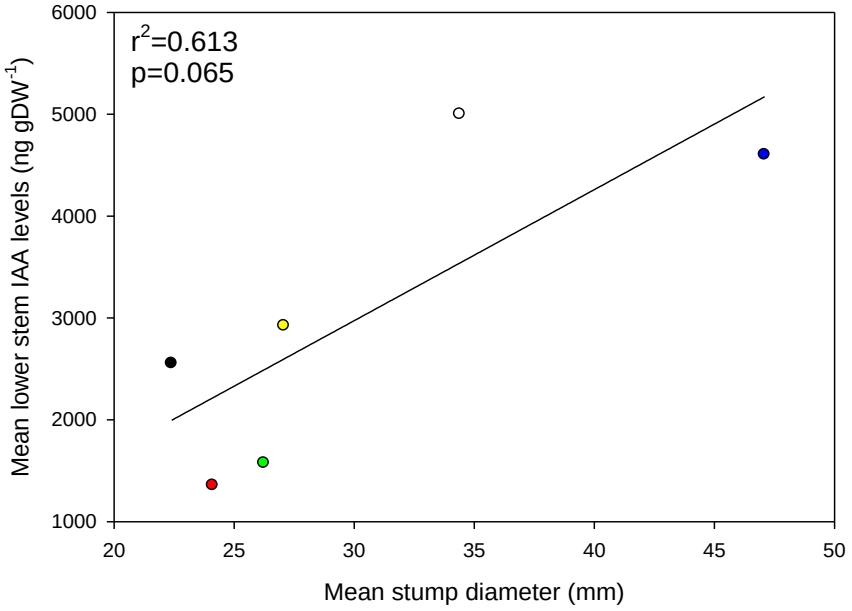


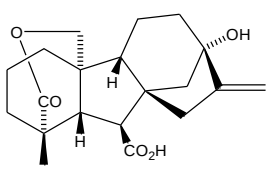


Indian Head SK 2006 Hybrids – Growing xylem tissue GA44 & IAA concentrations, each expressed as the mean of 3 ramets, were correlated, across 6 genotypes, with mean (of 3 ramets) tree stump diameters of those same genotypes

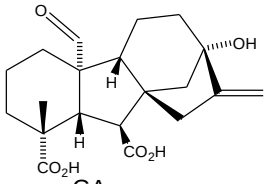
WP-69 →Tristis (white) → **Walker** → **Hill (yellow)** → **CanAm** →**Max (black)**



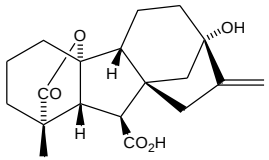




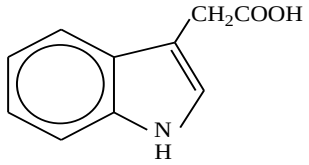
GA44



GA19



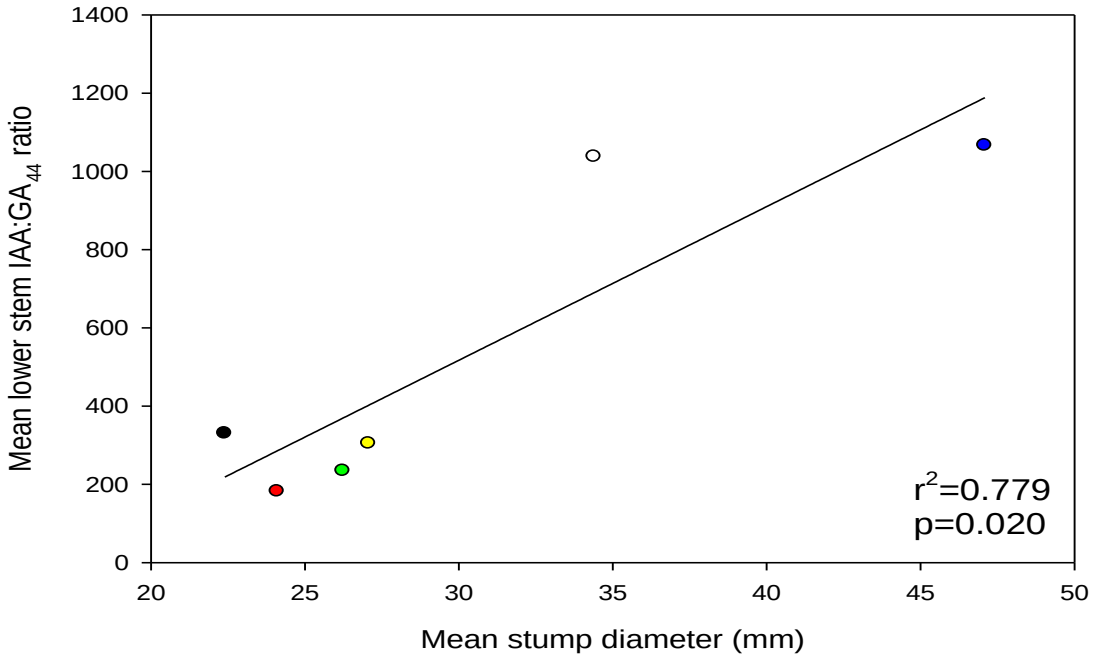
GA20

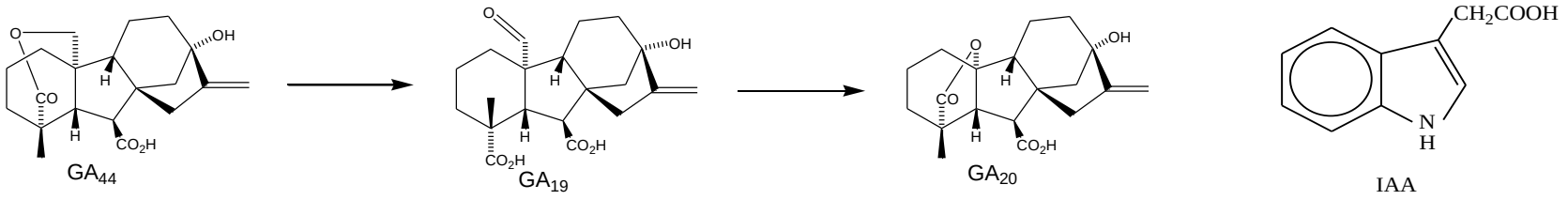


IAA

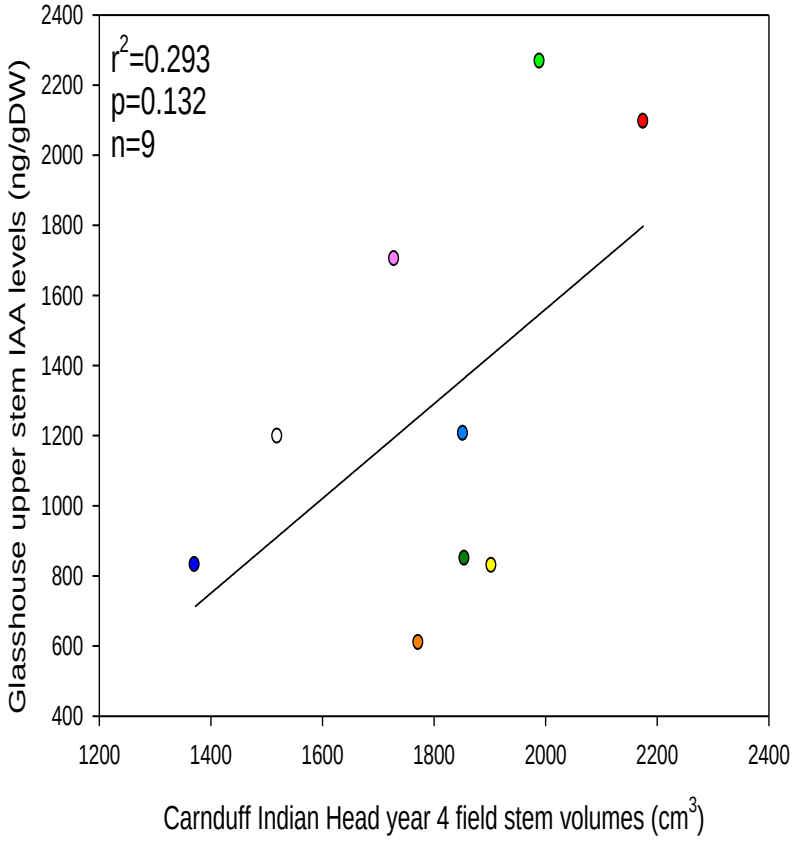
Indian Head SK 2006 Hybrids – Growing xylem tissue GA44 to IAA ratios, each ratio expressed as the mean of 3 ramets, were correlated, across 6 genotypes, with mean (of 3 ramets) tree stump diameters of those same genotypes

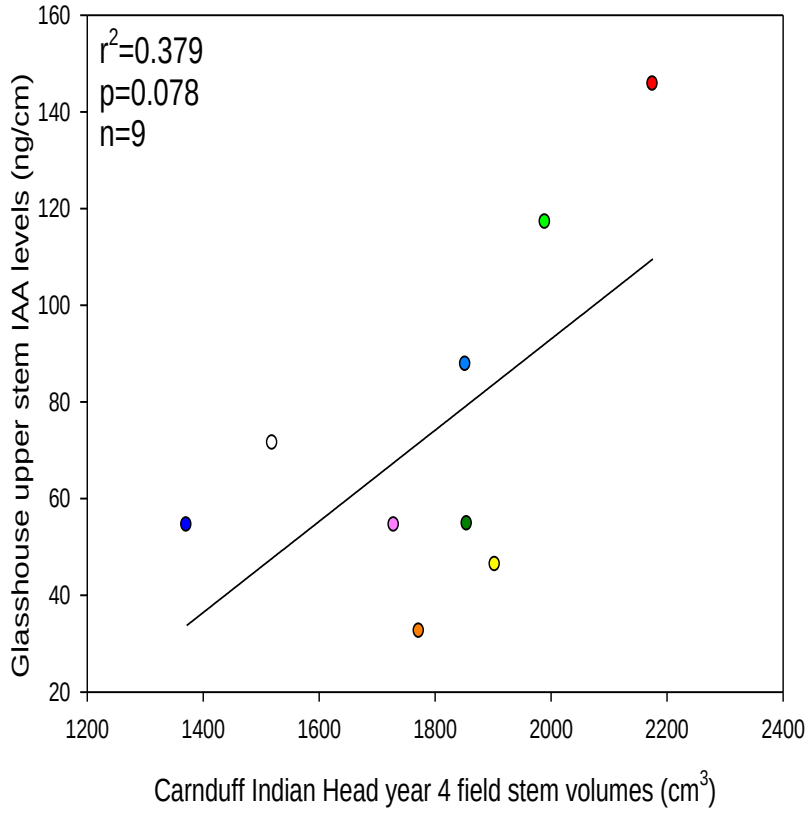
WP-69 →Tristis (white) → **Walker** → **Hill (yellow)** → **CanAm** →**Max (black)**



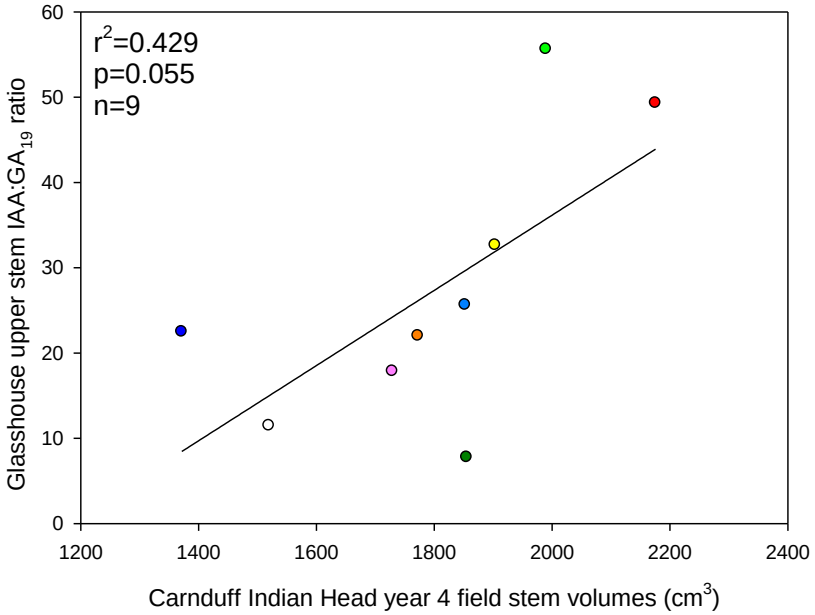


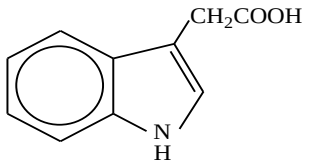
Balsam Poplar. Both GA1 and its catabolite, GA8 "failed" as markers of field performance across genotype. However, there were NEW correlations that appeared. Specifically, stem tissue IAA concentration (ng per g DW tissue, left & ng per cm lgth of internode tissue extracted, right).



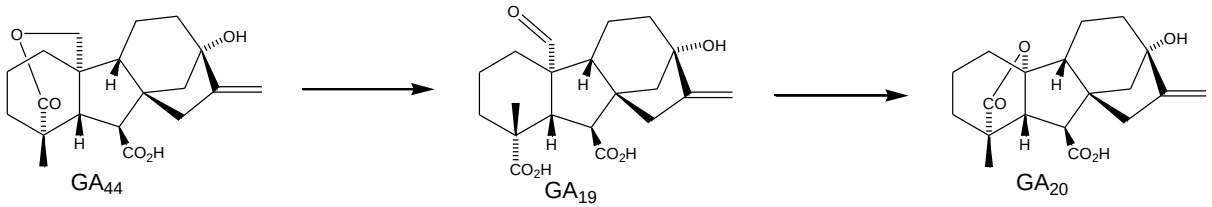


Balsam Poplar. Further, one of the NEW correlations (with field performance) that appeared was GA19, and especially, the ratio of IAA to GA19. . Thus, IAA:GA19 ratio, across genotype, showed a positive and near-significant correlation with tree stem volumes at Year 4 (2009) in a field provenance trial at Indian Head, SK.





IAA



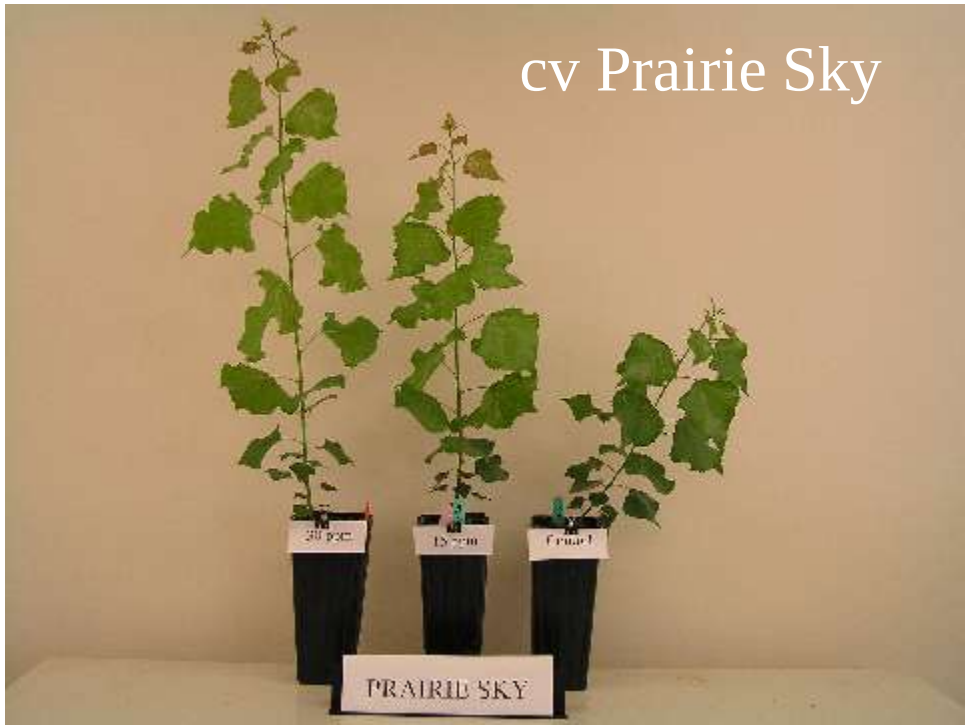
Conclusions: For poplar hybrids adapted to growing in Alberta and Saskatchewan, and also for balsam poplar, it is apparent that:

Growing tissue concentrations of GA1 and GA4 do NOT **reflect** inherent stem growth capacity "**in the field**" in the interior of Canada's prairie provinces.

Rather, it appears that "upstream" C20 gibberellins, i.e. GA44, GA19 & possibly GA20, whose biosynthesis is controlled by GA20 oxidases (& encoded for by *GA20ox*) may provide the "markers" for inherent stem growth capacity. Additionally, the auxin, IAA, which may regulate the activity of GA20 oxidase, or expression of *GA20ox*, may also be a useful "marker".

Finally, it should be noted that gibberellin biosynthetic steps controlled by GA20 oxidase (i.e. GA44 → GA19 → GA20) are under photoperiod control, with Short Days down-regulating the expression of *pttGA20ox*, as was shown for late expanding leaves of the aspen hybrid, *P. tremula x P. tremuloides* (Eriksson, M.E. & T. Moritz. 2002. Planta 214:920-930).

Both Tree Height and Diameter of the Slower-growing Hybrid Poplar cv. Prairie Sky Can Be Increased – Almost 3-fold by Applied Gibberellin



30 15 0 ppm

of soil-applied
GA_3

In Contrast, for the Exceptionally Fast-growing Hybrid Poplar cv. WP-69, the Height & Diameter Increases that Result from Applied Gibberellin Are Much Less, ca. 1.3-fold

30 15 0 ppm

of soil-applied GA3

Two-Year Old Willow (*Salix aquatica*) on Peat soil in Finland – 30 Tonnes of Dry Biomass per Hectare