

Introduction: A recent publication from Banerjee et al. 2010, reported differences in corn stover glucose release ranging from 64 to 95%. These significant differences in fermentable glucose levels would likely result in tremendous variability in bio-refinery ethanol yield from corn stover feedstock. Preliminary GLBRC analyses of corn stover (cultivar Pioneer 36H56) showed year to year (2008-2009) variability in ethanol precursors, glucose and free sugars, suggesting a significant environmental effect on stover quality. Additionally, work published by Saxena and Stotzky (2001) showed that the lignin content, an anti-quality agent for ethanol production, was significantly higher (33-97% higher) for Bt lines compared to their respective non-Bt isolate.

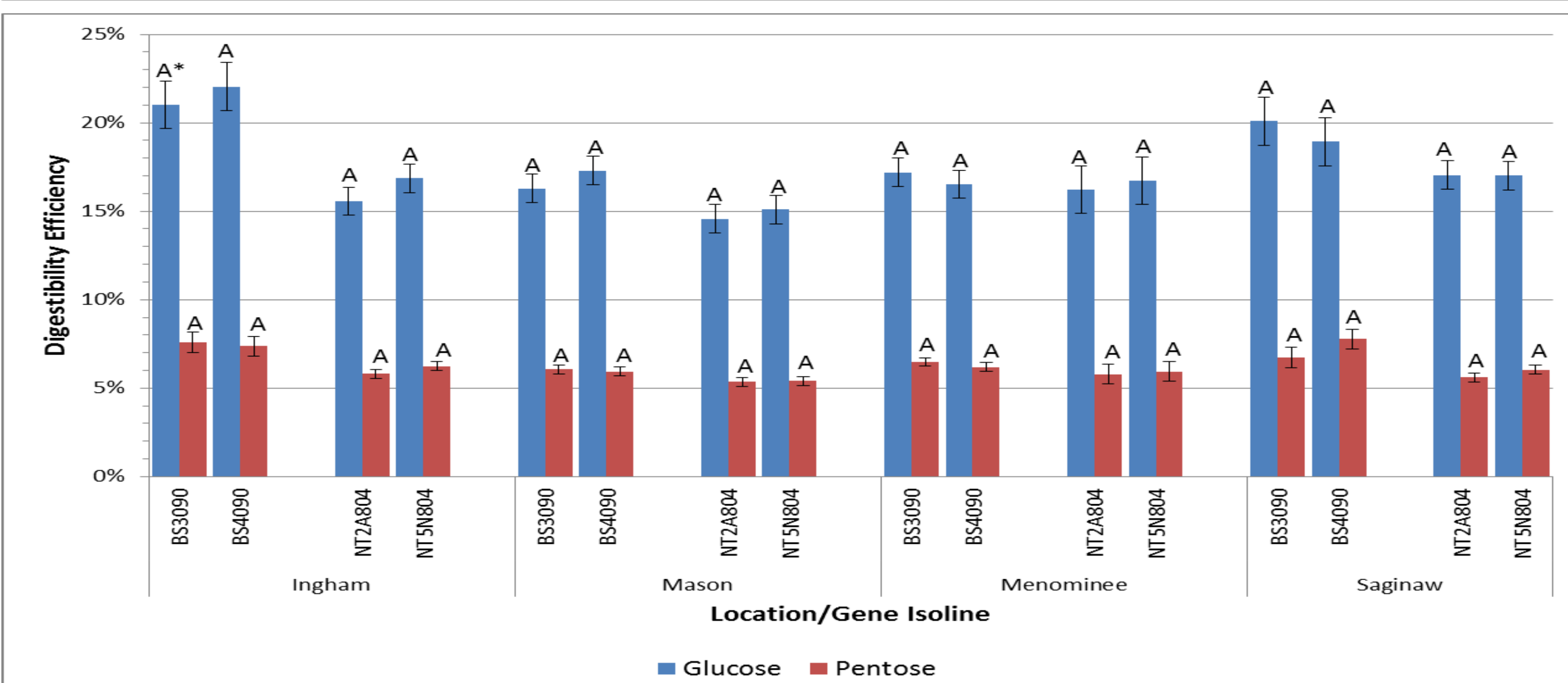
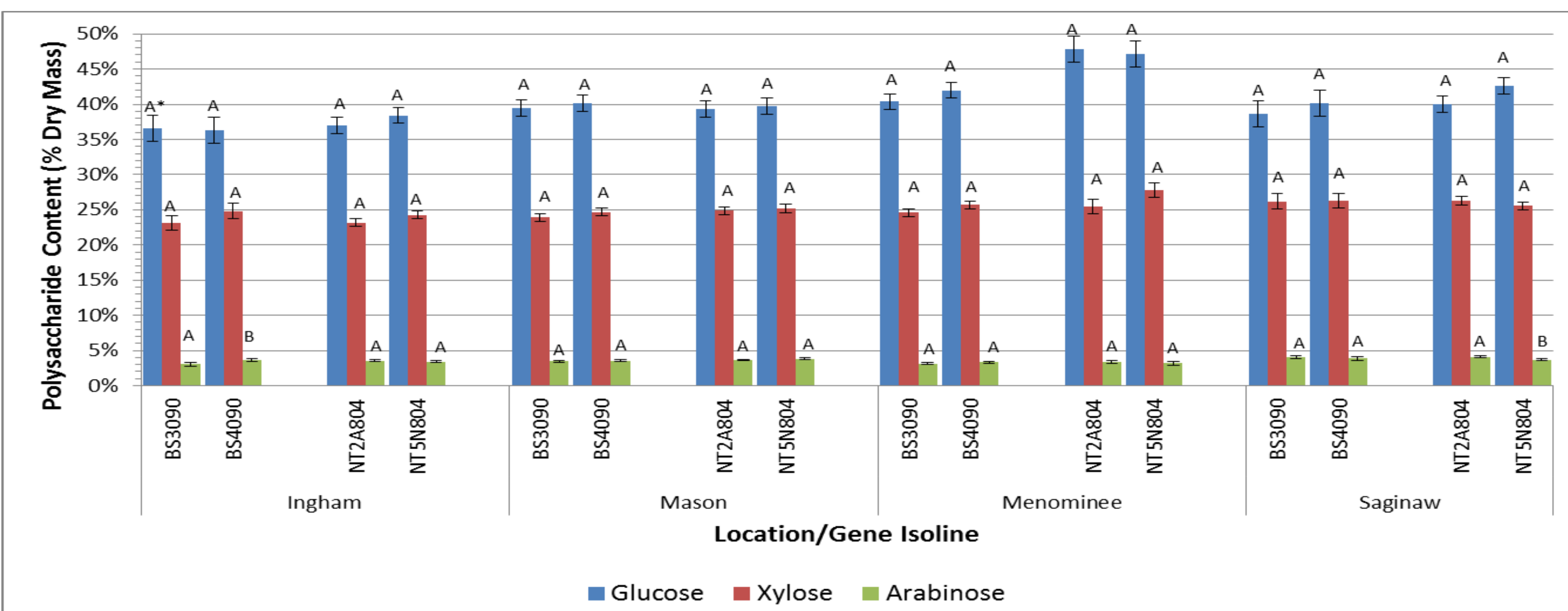
Objective: To evaluate the effect of the Bt trait, location and hybrid differences on corn stover and cob ethanol yield.

Materials & Methods: For the experimental design, 2 hybrid pairs (one Bt and one near-isoline relative) were analyzed giving a total of 4 hybrids. Each hybrid pair were grown at two latitudinal differing locations at the MSU Hybrid trials in four replicated plots. At each location, the entire corn plants were harvested. Then the ears were removed from the plants and the grain was shelled from the cob. Wet and dry weights were recorded from all three fractions for each plot (stover, cob, and grain). This experiment was repeated in the years 2010 and 2011. Samples were ground and were submitted for AFEX pretreatment. Samples were analyzed for glucose, sugar profile, cellulose, and IVTD. A sub-set of 16 samples were fermented and analyzed for ethanol yield. All data was analyzed using the PROC TTEST procedure in SAS 9.2. Bt trait and isolate pairs were tested for the different locations and in both the years.

Results:

- *Effects were considered significant at $P < 0.05$
- Mean separation between variables was obtained by LSD Test
- Samples were tested for composition, lignin and IVTD
- Samples were tested for composition, fermentation, and ethanol yield
- Component levels did not vary when stover was subjected to biorefinery simulated pretreatments of weak acid or AFEX followed by enzymatic hydrolysis.

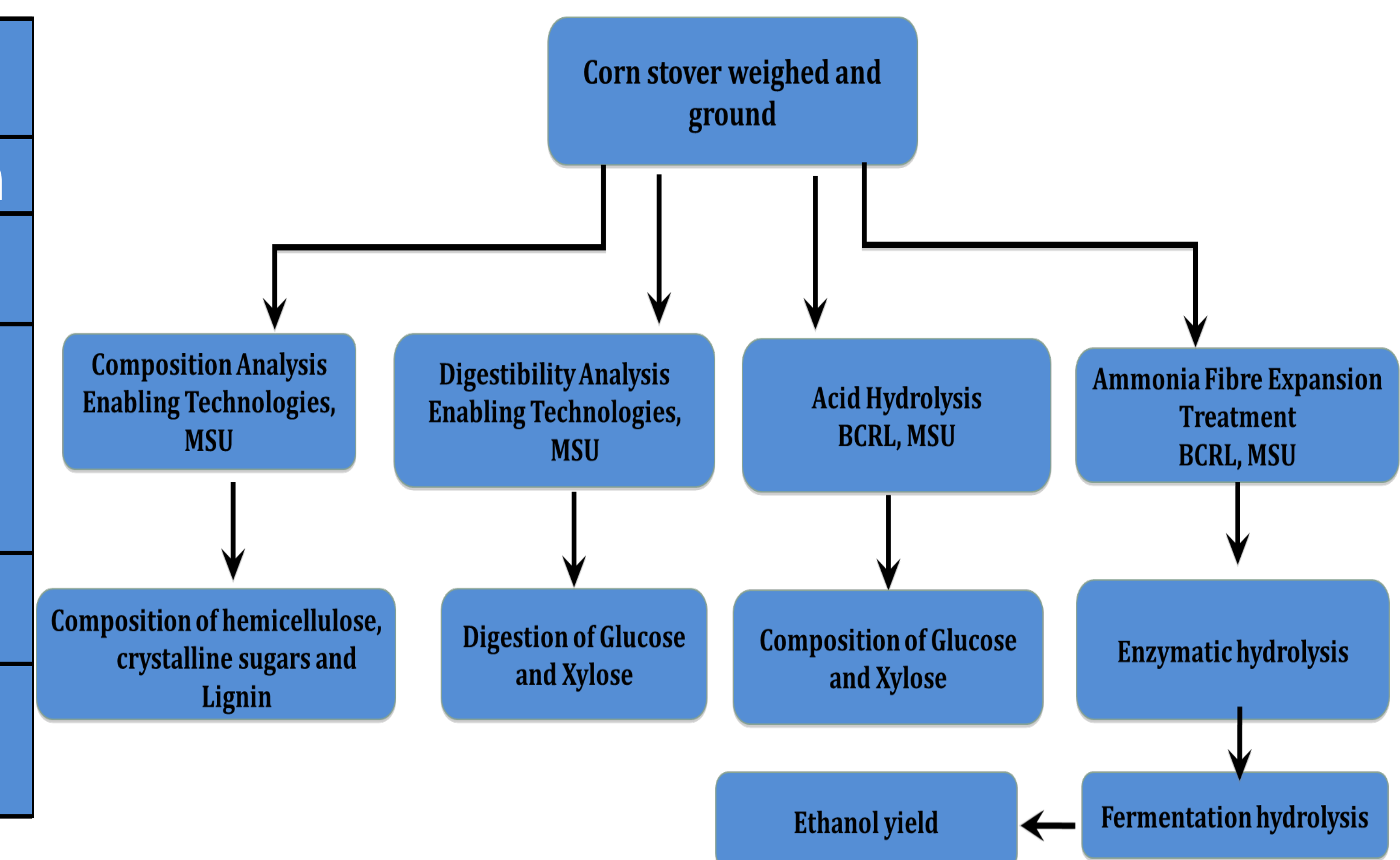
Comparison of Bt. vs. Non-Bt Corn Using Enabling Technologies



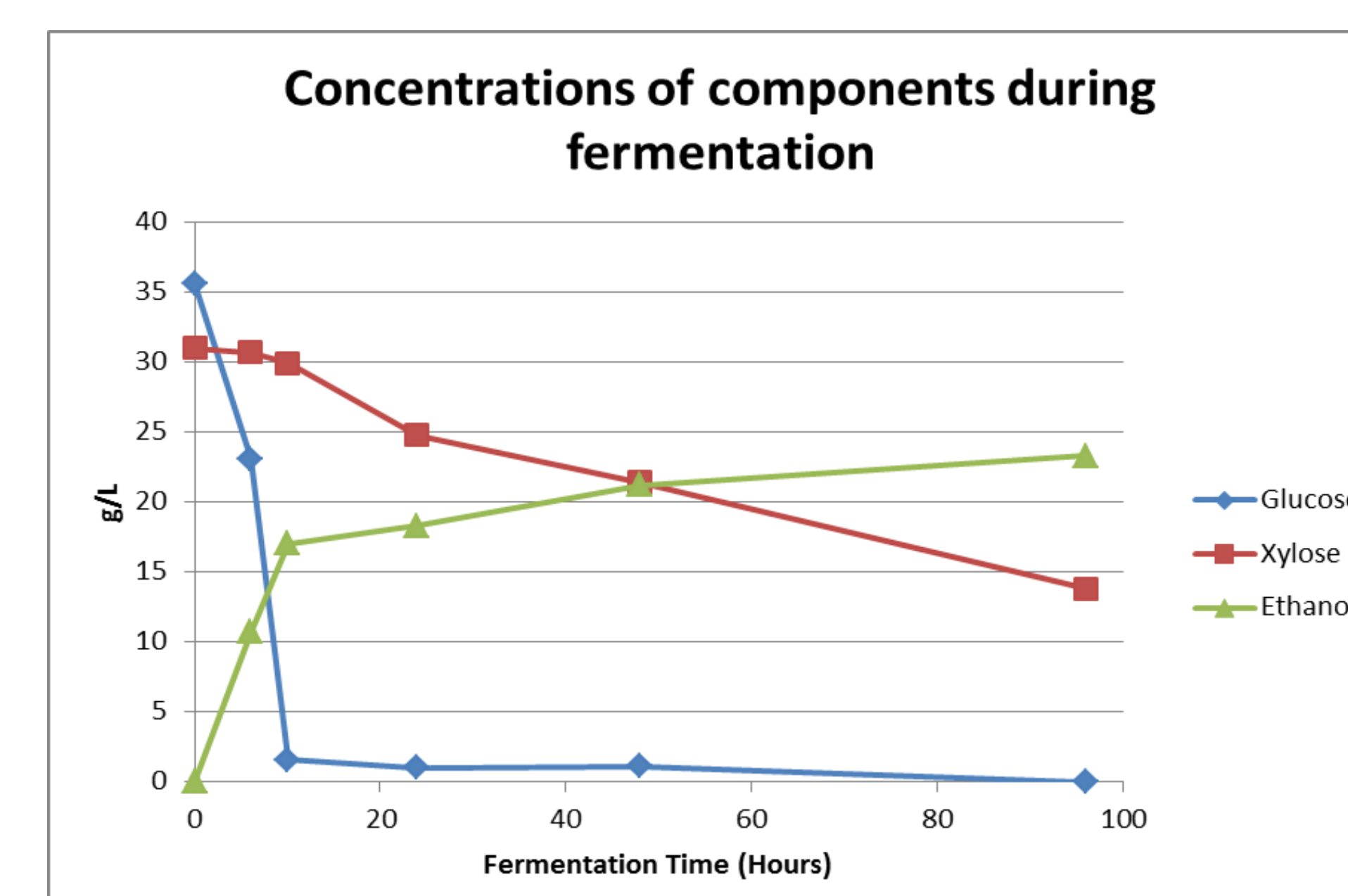
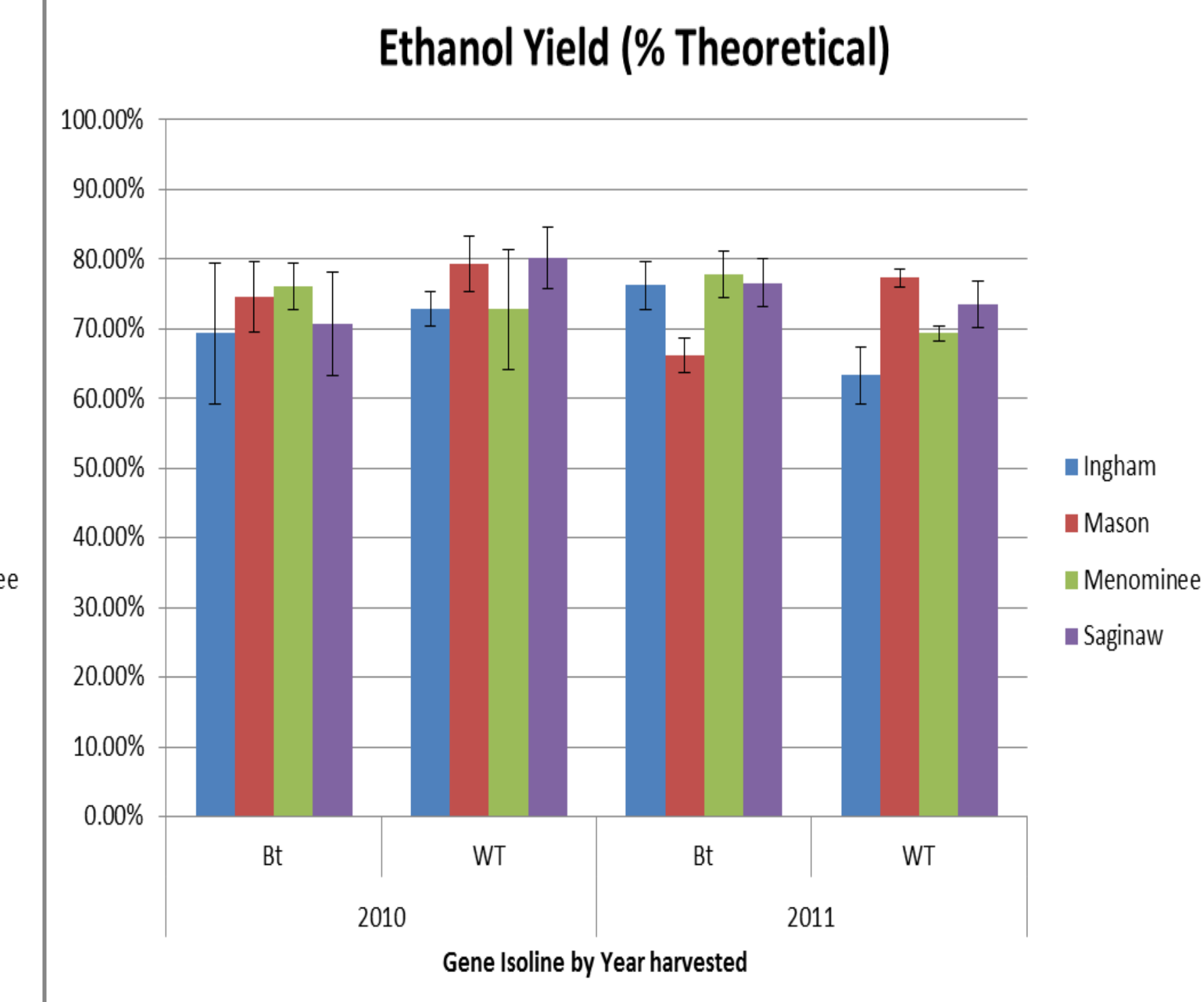
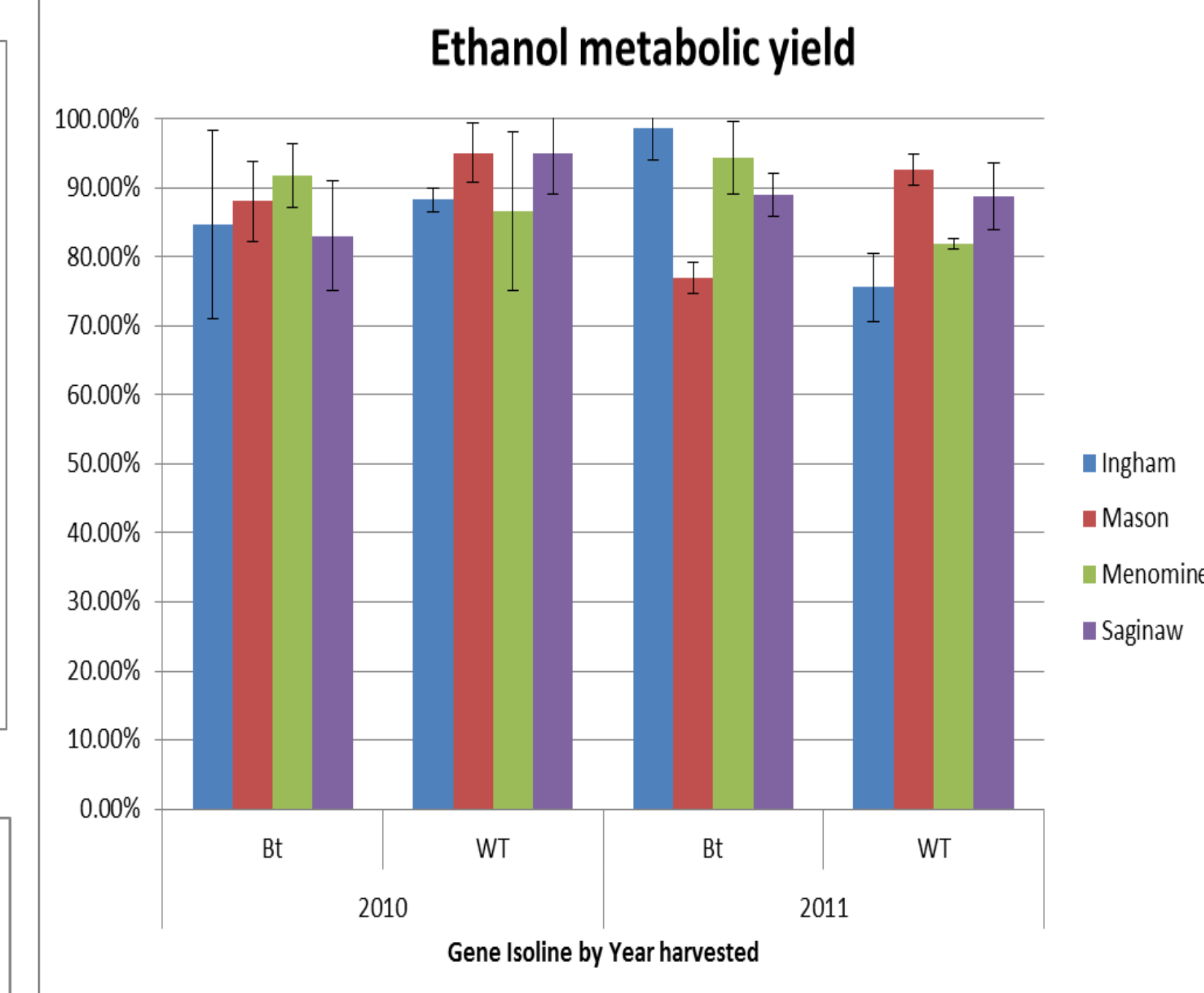
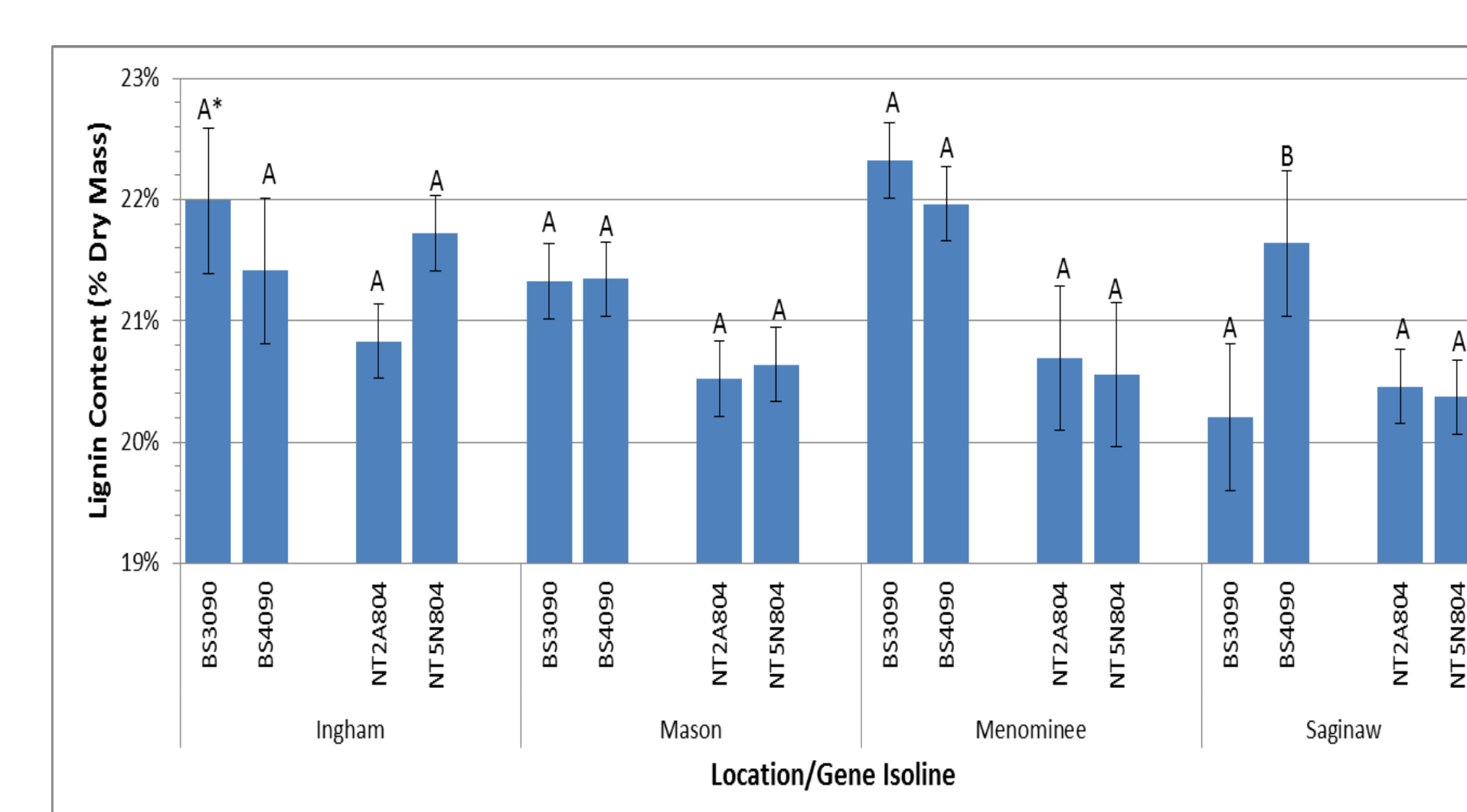
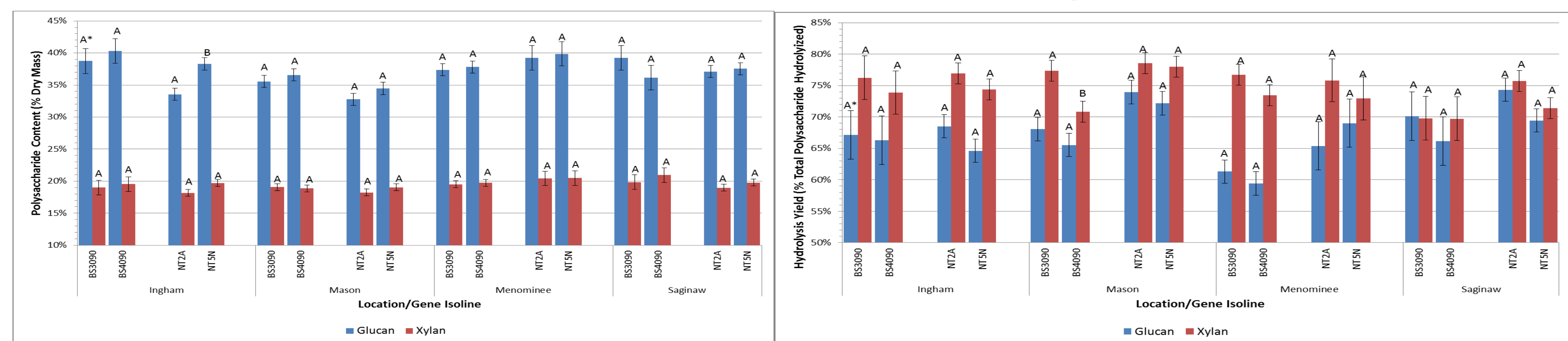
Sample Collection

Isoline	Bt trait	2010				2011			
		Ing	Sag	Mas	Men	Ing	Sag	Mas	Men
NuTech 2A804	No	✓	✓	✓		✓	✓	✓	✓
NuTech 5N804 GT/CB/LL/RW	Cry1AB & mCry3	✓	✓	✓		✓	✓	✓	✓
Bayside 4090	No			✓	✓	✓	✓	✓	✓
Bayside 3090 GT/CB/LL	Cry1AB			✓	✓	✓	✓	✓	✓

Flow Chart from Corn Stover to Ethanol



Comparison of Bt. vs. Non-Bt Corn Using BCRL



Conclusions:

- With the lone exception of the NuTech isolate pair at the Ingham location, the presence of the Bt Cry1AB & mCry3 transgene in corn stover did not affect the concentration of the important ethanol quality components glucose and xylose.
- Hybrid did not have an effect on the corn stover compositional analyses of ethanol quality component glucose and anti-quality component lignin with an exception in one isolate pair in Saginaw.
- Subset of Corn stover samples tested for compositional levels of glucan, xylan and lignin did not have a significant correlation ($R^2 = 0.011$) on the theoretical ethanol yield.
- No significant effects were observed in between location, hybrids and Bt. trait

References: Banerjee, G., S. Car, J. S. Scott-Craig, M.S. Borrusch, N. Aslam, and J. D. Walton (2010) Synthetic enzyme mixtures for biomass deconstruction: production and optimization of a core set. *Biotechnol. Bioengineer.* 106:7 07-720.
Saxena, D. and G. Stotzky. 2001. Bt corn has a higher lignin content than non-Bt corn. *Amer. J. Bot.* 88(9):1 704-1706.
Gao, J.*, Qian, L., Thelen, K. D., Hao, X., Sousa, L.D., Lau, M. W., Balan, V. and Dale, B.E., (2011) Corn harvest strategies for combined starch and cellulosic bioprocessing to Ethanol. *Agronomy journal.* 103 (3): 844-850.