Industrial Hemp: Crop for the future?

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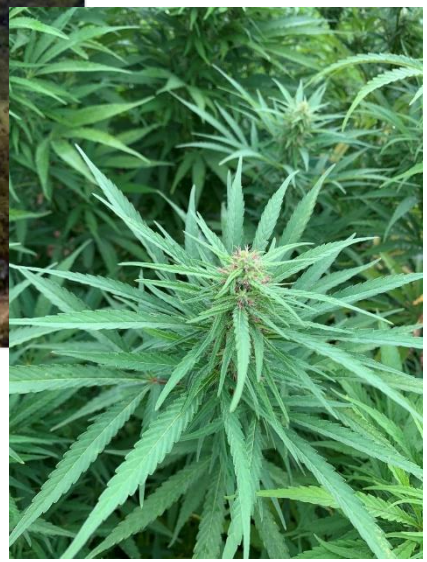

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The Connecticut Agricultural Experiment Station
Analytical Chemistry Department

Accredited to ISO 17025 by A2LA since 2016
Pesticide Residues, Aflatoxins and Arsenic in Food

Committed to expanding our scope of accreditation to hemp testing in fall of 2020

Testing this summer followed the same quality management system as our accredited programs

Agricultural Improvement Act 2018 Definitions

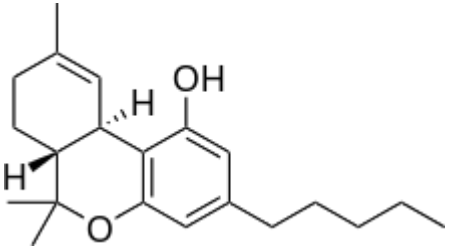
1. Sec. 297A Definitions – "HEMP.—The term 'hemp' means the plant Cannabis sativa L. and ***any part of that plant***, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a ***delta9 tetrahydrocannabinol*** concentration of not more than 0.3 percent on a ***dry weight basis***."

2. Sec. 297B. State and Tribal Plans requires: "a procedure for testing, using ***postdecarboxylation*** or other similarly reliable methods, delta-9 tetrahydrocannabinol concentration levels of hemp produced in the State or territory of the Indian tribe"

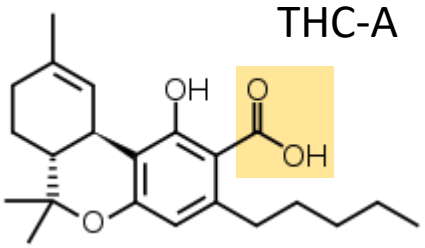
3. Two critical points for testing:
    1. Analysis of THC includes THC-A
    2. Dry weight is not defined

4. Our lab decided to test total delta-9 THC using gas chromatography with flame ionization detection. Testing is quick and easy providing total THC in one step. Testing includes analysis for CBD that includes CBD-A

Method Considerations



THC



THC-A

- Quick turn around
  - Only two weeks from sample to harvest
- Total delta-9 THC for compliance with farm bill
  - Includes THC-A (new for 2018)
- Report specifies “pass” if <0.3%, or “fail” if >0.3%
  - Decision includes the measurement uncertainty
  - New USDA guidance states MU *must* be on the report
- Measurement Traceability
  - Is the lab getting the same result regardless of time or analyst?
  - Is the lab getting the same result as other labs?

ISO/IEC 17025

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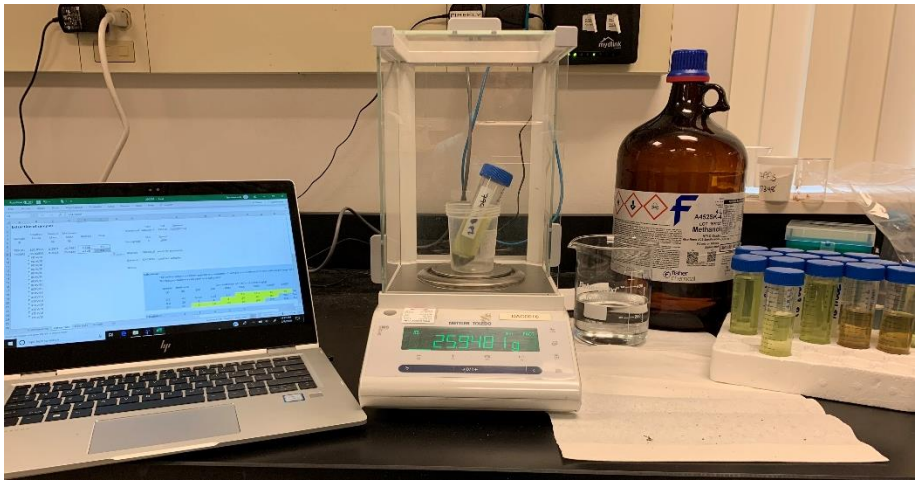
Overview of Testing

Dry sample overnight in oven at 90 ^{o}C

Extract 0.2 grams of sample into 25 grams of methanol

Inject Gas Chromatograph-Flame Ionization Detector

run time is 10 minutes



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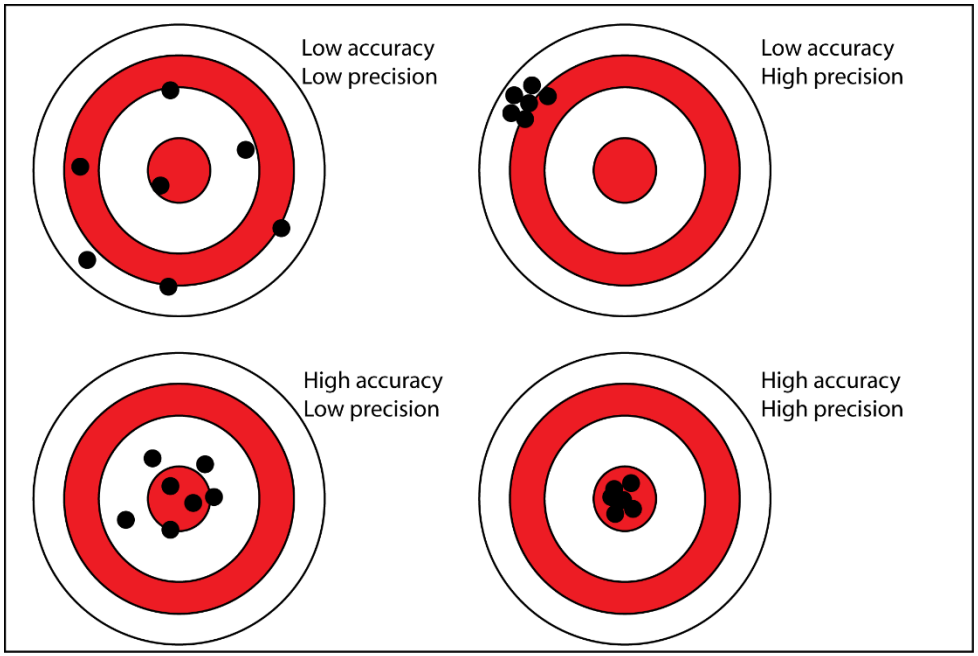
Measurement Uncertainty
Affected by both accuracy and precision

Accuracy: How close a measurement is to the true value

Precision: How close two or more measurements are to each other



Quality Control

- Certified reference material with each daily run
- Blank (oregano) with each daily run
- Samples run in duplicate
- Successful completion of Proficiency Testing

HEMP
Proficiency Testing Program

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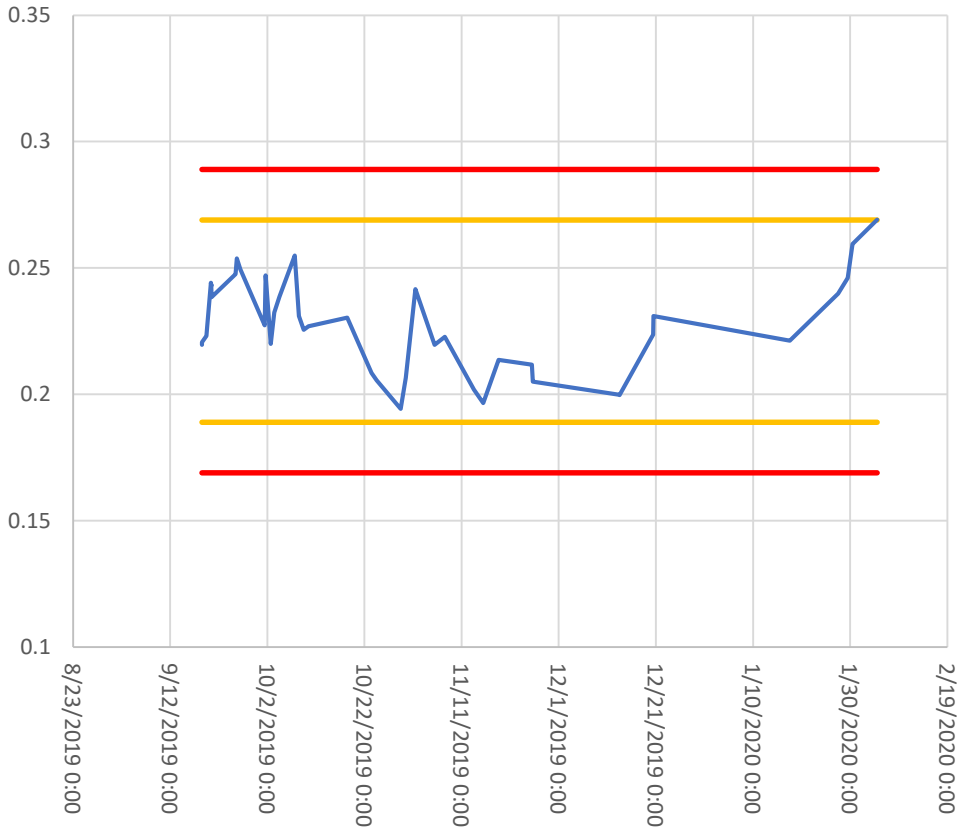
Ensuring Accuracy and Precision in our Testing

Ran certified reference material with each batch of samples



Certified value = 0.194%
Lab value = 0.227%

This particular material has an interference resulting in a bias in our result, about 117% of the true value

Same interference noted in about 8% of submitted samples

Standard deviation = 0.019%
95% confidence interval = 0.170% to 0.284%

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Proficiency Test Results 2020

Dried hemp from Kentucky

Sample ID	True Value	Lab value	Z-Score (3 to -3)	HorRat(r) (0 to 4.9)
Sample 1	0.284%	0.287%	-0.06	0.48
Sample 2	0.136%	0.144%	-0.27	1.06
Sample 3	0.300%	0.300%	0.0	0.24
Sample 4	0.0686%	0.0653%	-0.24	1.34

Z-score is a measure of closeness to the true value (accuracy)
HorRat(r) is a measure of how close the three reported lab values are to each other compared to the expected difference based on data from other labs (precision)

Our lab is getting the same result as other labs

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Lockwood Farm Plot

- Grew five varieties:
  - Cherry 307, Cherry 308, Youngsim10 lot “2018WFS1” and Youngsim10 lot “2018WFS17A” and Z-1 (Tried germinating hemp seed purchased on Amazon, but no germination)
- Started from non-feminized seed in greenhouse, planted in field on Friday, June 21, 2019
- 5 Rows spaced 5 feet apart, plants spaced 2 feet apart
- Used black plastic to control weeds within the rows and hose irrigation under the plastic

Lockwood Farm Plot
June 28, 2019

Probably root rot

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Lockwood Farm Plot
July 22, 2019

Maybe female??

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Lockwood Farm Plot
August 20, 2019

Corn borer

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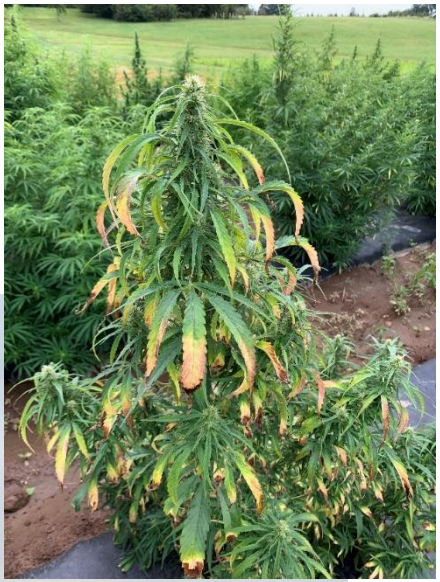
Lockwood Farm Plot
Sept 10, 2019



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Lockwood Farm Plot
October 4, 2019

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Summary of Growing Experience

1. Youngsim 10 varieties bloomed first beginning around end of July and continuing for about three weeks

2. Started culling males the first week of August, but by then they were in full bloom and loaded with native bees (but not honeybees)

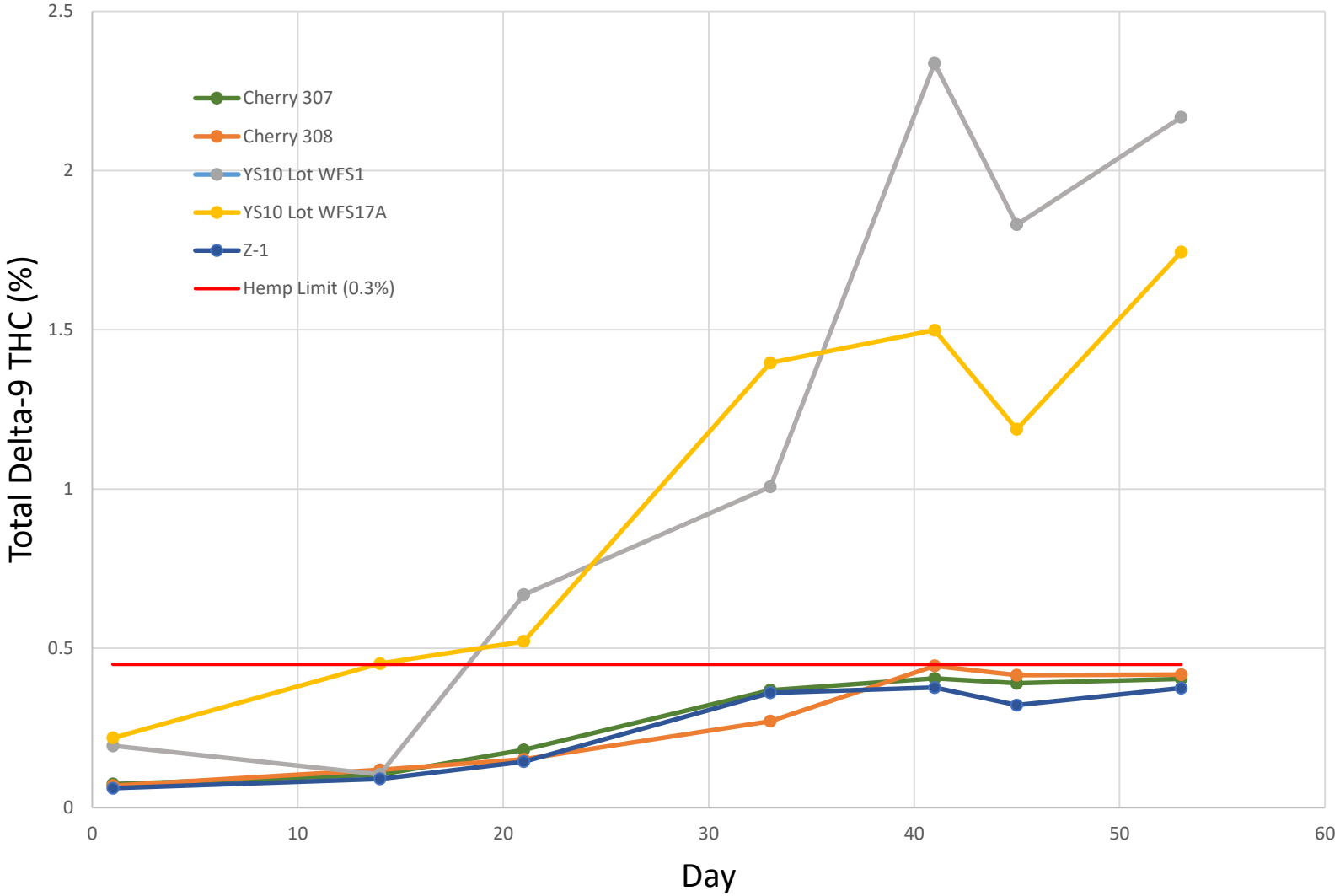
3. The other three varieties started blooming around the second week of August

4. Blooming continued in all varieties until the end of August!

5. The Z-1 variety was about 25% female, others were roughly 50% female

6. Plants subject to corn borer, but also probably viral infection, as well as root rot

Total Delta-9 THC testing Results



Sampling Started August 16 and ended on October 8

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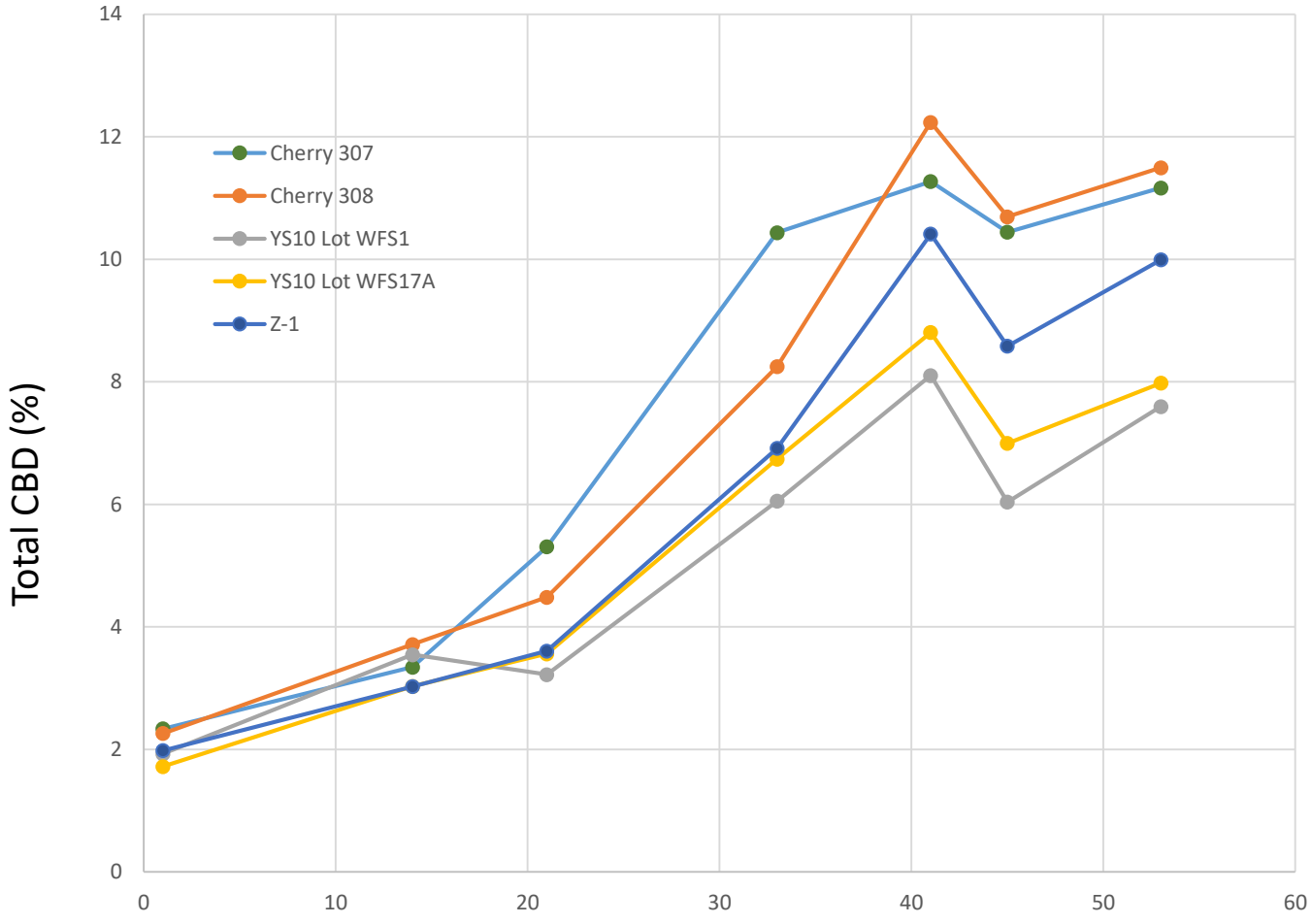
Total CBD testing Results



Sampling Started August 16 and ended on October 8

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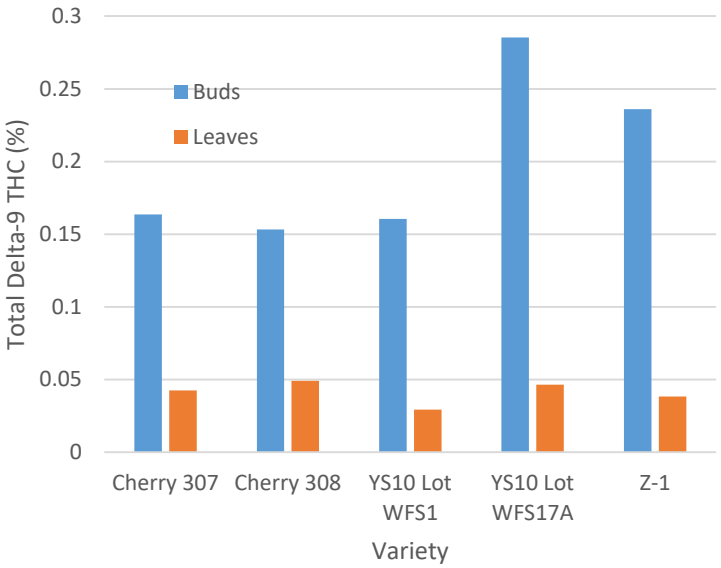
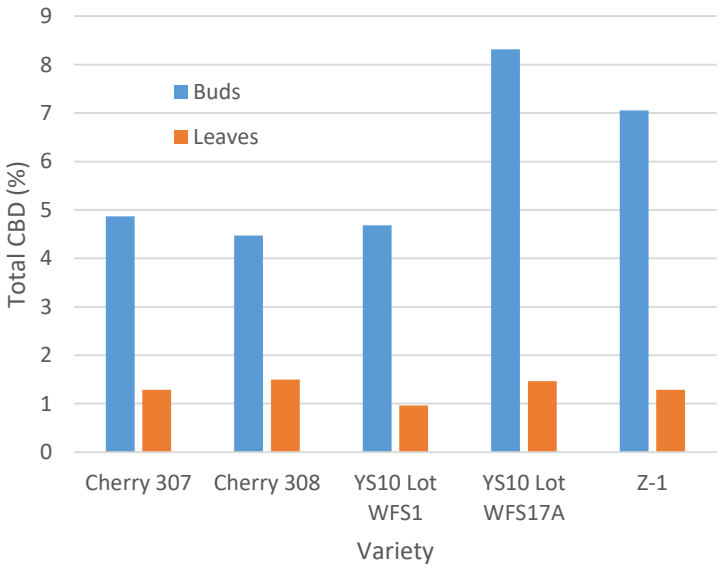
Leaf Material versus Bud Material

Collected on September 7, 2019, dried and separated





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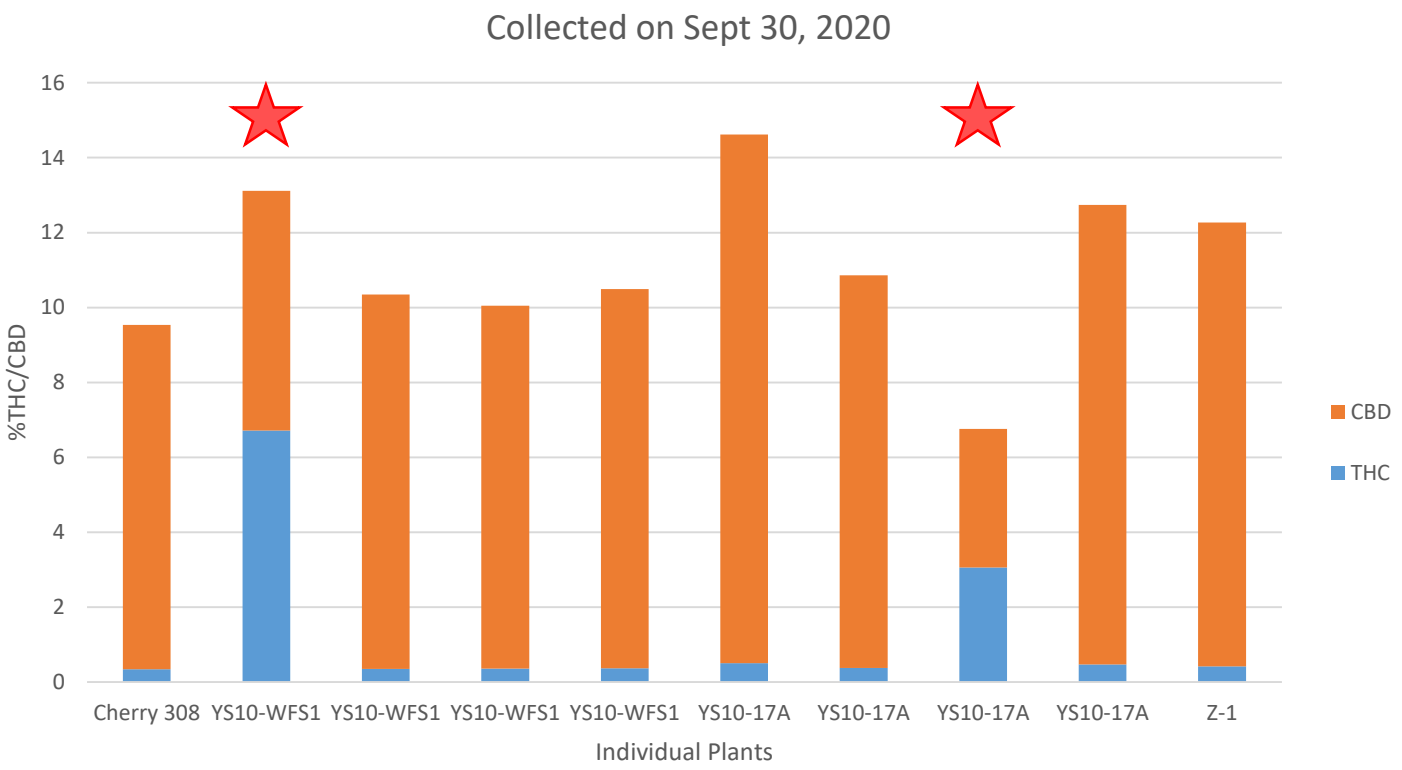
Test of Individual Plants



Only two of eight plants of the Youngsim10 variety failed the THC test 6.7% and 3.1% THC

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High Pressure Liquid Chromatograph (HPLC)

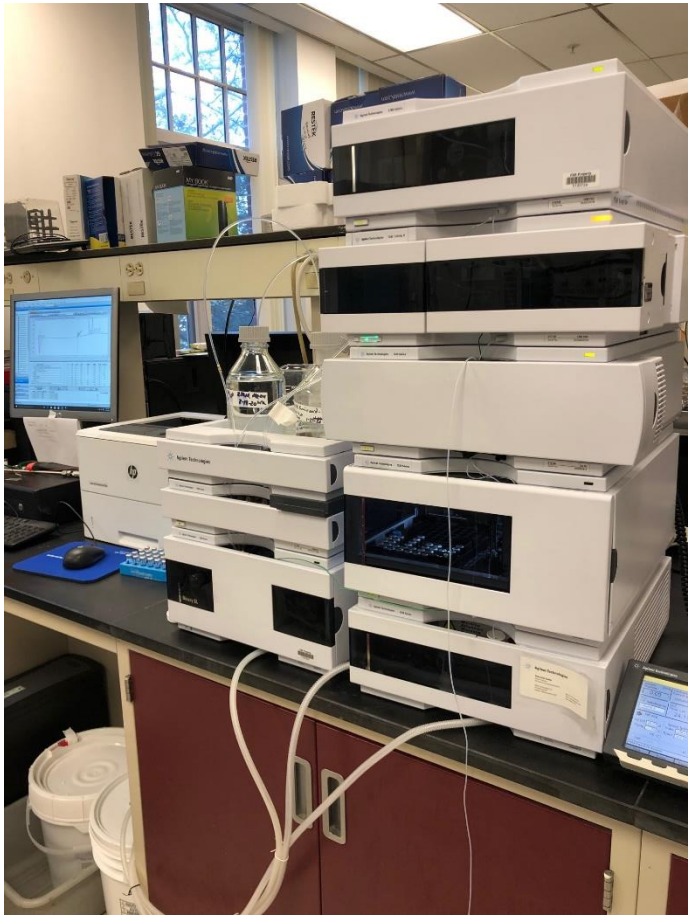
Solvents

High Pressure Pump

UV and FLD Detectors

Column (Separation)

Samples (Injector)



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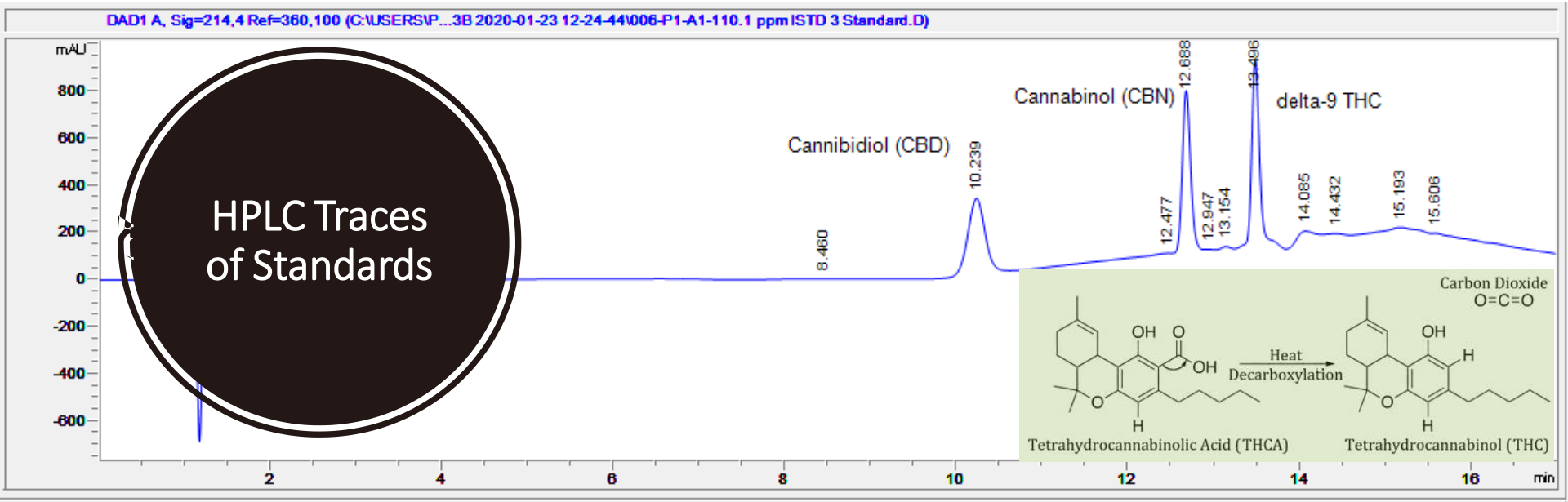
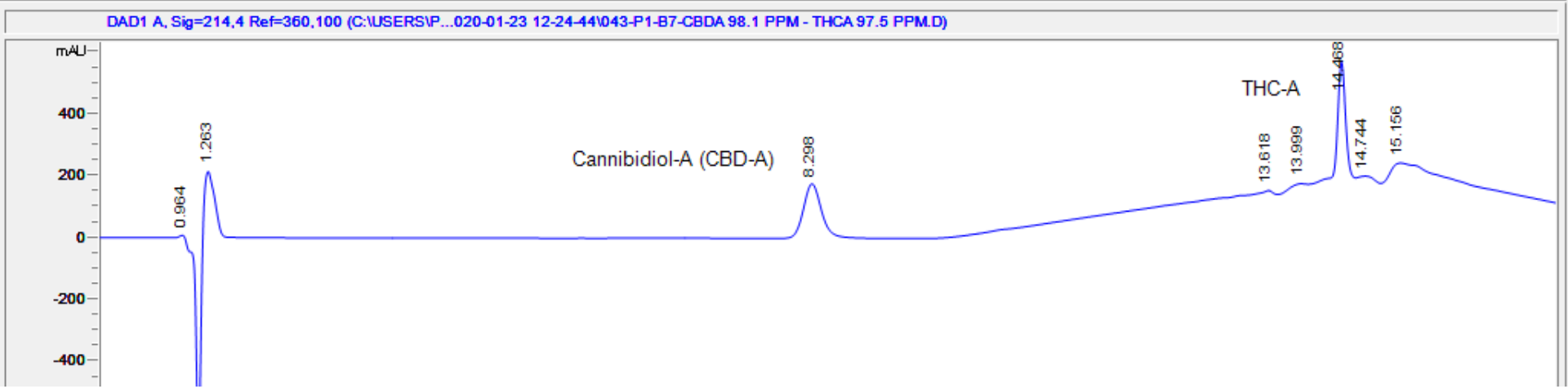
HPLC Traces of Standards





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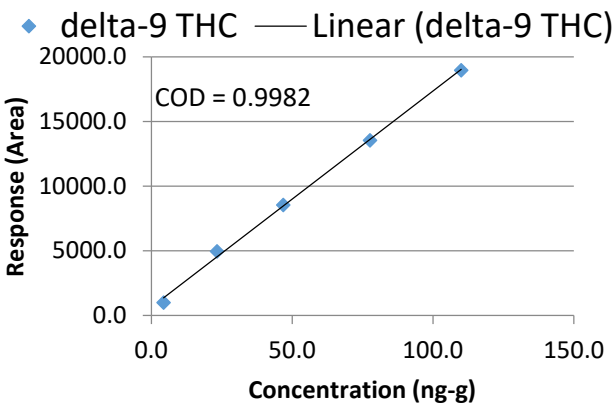
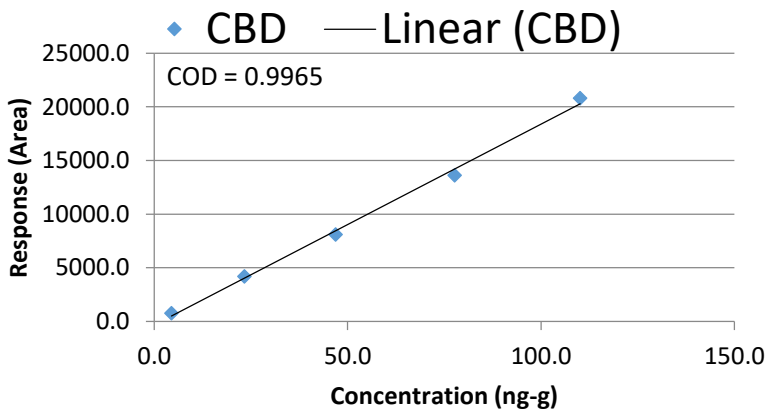
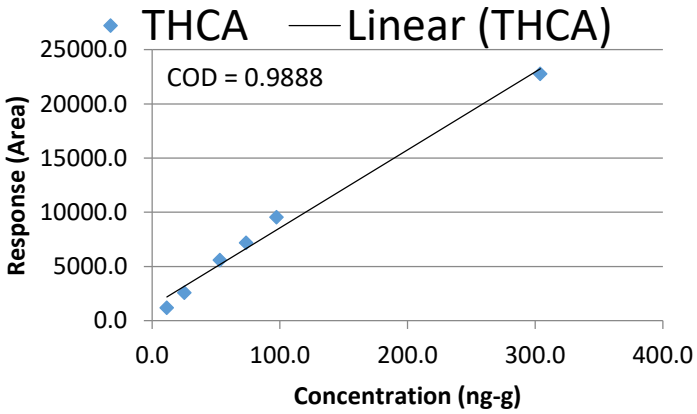
Linearity of Analysis 4 ppm – 100 (300) ppm







Standards of THCA / CBDA and THC / CBD are prepared Separately and monitored for degradation

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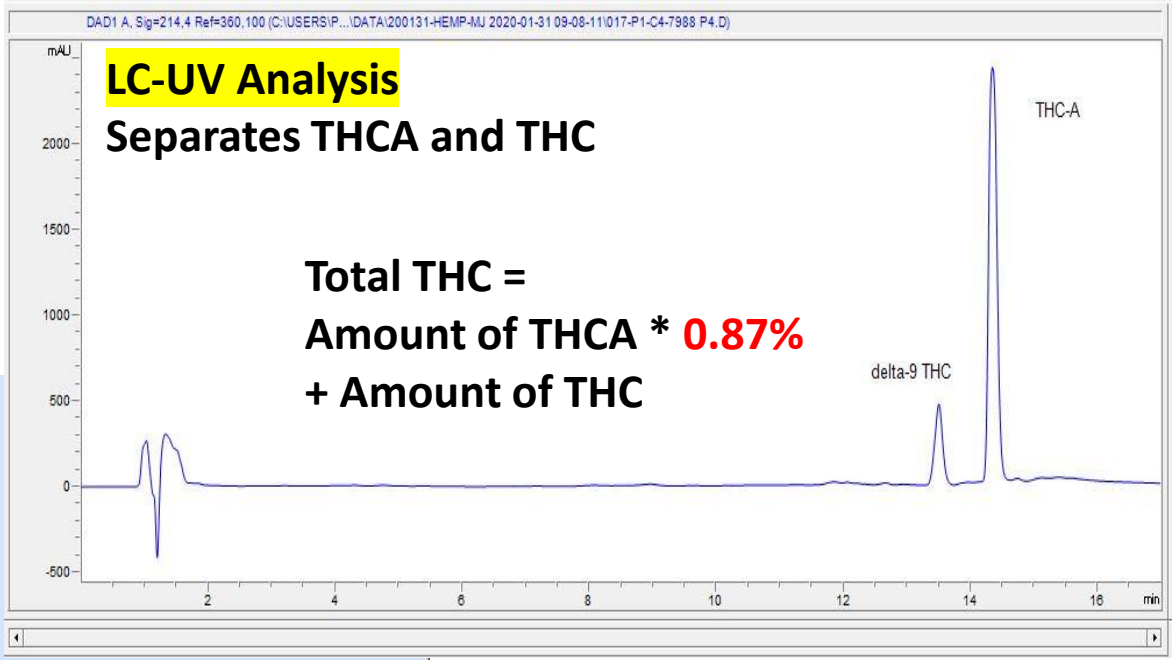
MeOH

Soak

LC-UV Analysis

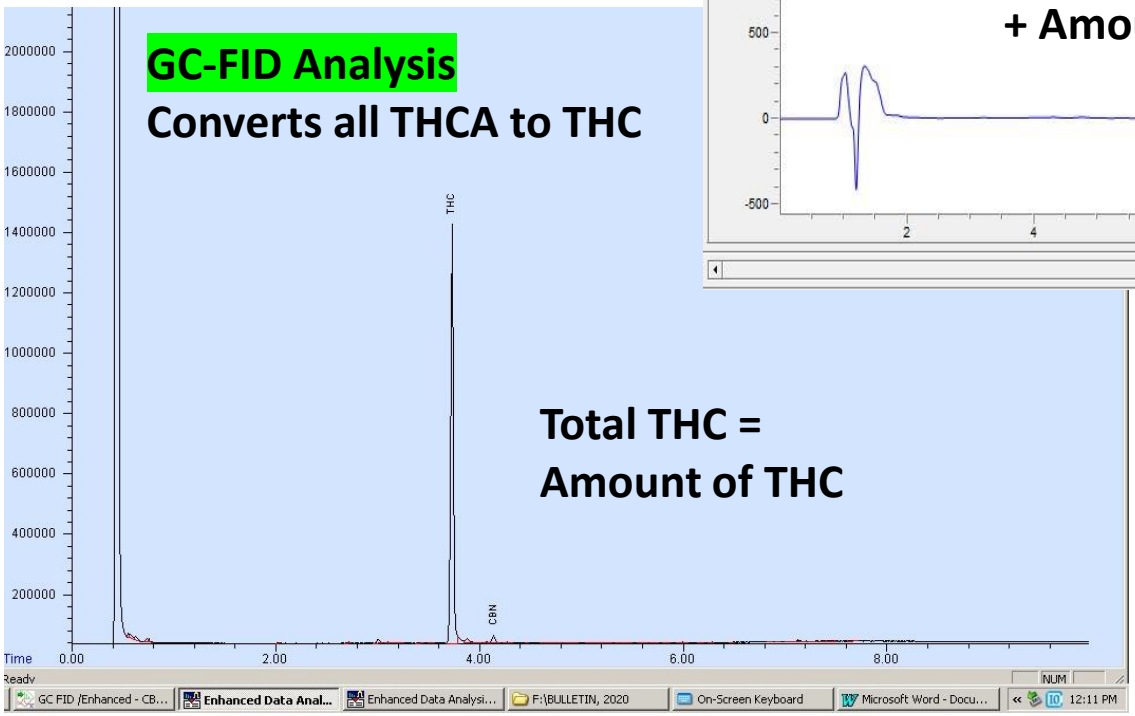
Separates THCA and THC



Total THC =
Amount of THCA * 0.87%
+ Amount of THC

GC-FID Analysis

Converts all THCA to THC



Total THC =
Amount of THC

delta-9 THC by LC-UV = 6.1%

delta-9 THC by GC-FID = 5.6%

RPD Between Methods = 8.5%

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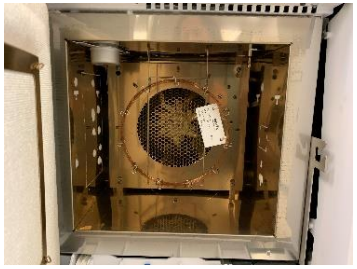
Conclusions

- Gas Chromatography with flame ionization detection is a robust method for analysis of total Delta-9 THC
  - Quick sample run time, robust and reliable
  - Virtually no maintenance on the instrument, no down time
  - Results between GC and LC are equivalent



- CBD and THC began to spike around the beginning of September
  - Early testing of THC levels may not be indicative of later test results

- Hemp varieties appear to have higher variability and poorer predictability than other plant varieties
  - Plants look different
  - THC levels different within the variety
  - *Both Youngsim 10 varieties failed the total delta-9 THC testing*

- The lack of definition of “dry weight’ may lead to significant differences in test results
  - This is potentially a significant source of difference between test results from different labs

Acknowledgements

Staff of the Experiment Station
- Dr. Jason C. White
- Ms. Kitty Prapayotin-Riveros
- Rich Cecarelli

Thank You!!

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