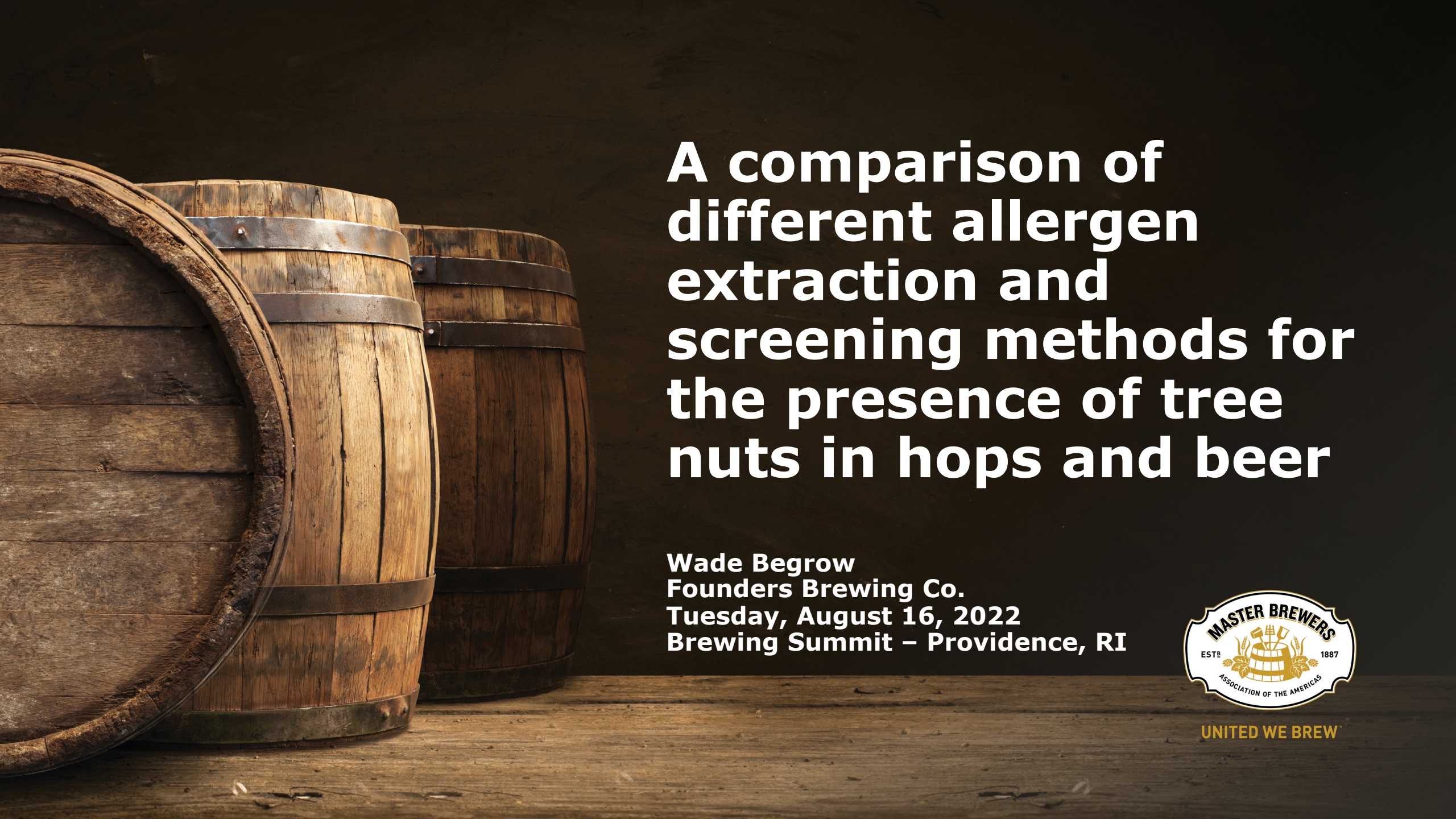


A comparison of different allergen extraction and screening methods for the presence of tree nuts in hops and beer

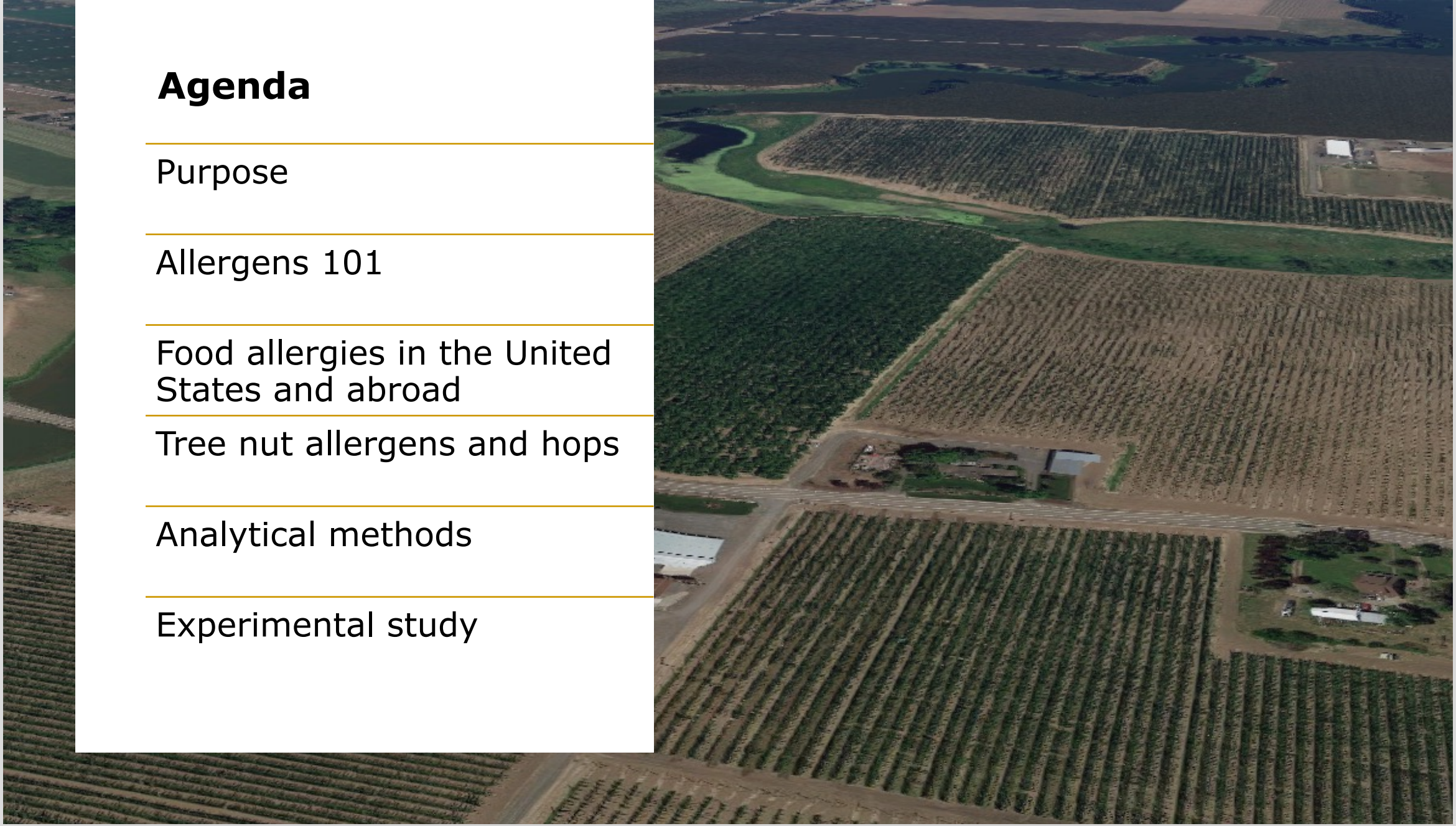
Wade Begrow
Founders Brewing Co.
Tuesday, August 16, 2022
Brewing Summit – Providence, RI

MASTER BREWERS ASSOCIATION OF THE AMERICAS EST. 1887

UNITED WE BREW™

Agenda

- Purpose
- Allergens 101
- Food allergies in the United States and abroad
- Tree nut allergens and hops
- Analytical methods
- Experimental study



Purpose

- Why is a microbiologist presenting about allergens?
- Why are allergens important for beer producers and consumers?
  - Food allergies affect about 4% of adults and 8% of children in the United States[1]
  - Peanuts and tree nut allergies are most common, but it varies by region
- Allergen labeling is important and can be tricky
- Various detection methods

Allergens 101

What is an allergic reaction?[2]

- A type I allergic reaction occurs when:

1. Allergen (antigen) enters body
2. IgE antibodies bind to allergen
3. Immune system releases antibodies
4. Release of histamine and inflammatory response

Food allergies

In the United States and abroad

Food Allergens in US and EU[1]

USA (FALCA)

- Egg
- Fish
- Milk / dairy
- Peanut
- Sesame
- Shellfish
- Soy
- Tree nuts
- Wheat
- Sulfites

EU

- Celery
- Egg
- Fish
- Gluten
- Lupin
- Milk / dairy
- Mustard
- Peanut
- Sesame
- Shellfish (including mollusks)
- Soy
- Tree nuts
- Sulfites

Tree Nuts and Hops

Tree Nuts (emphasis on hazelnut) and Hops

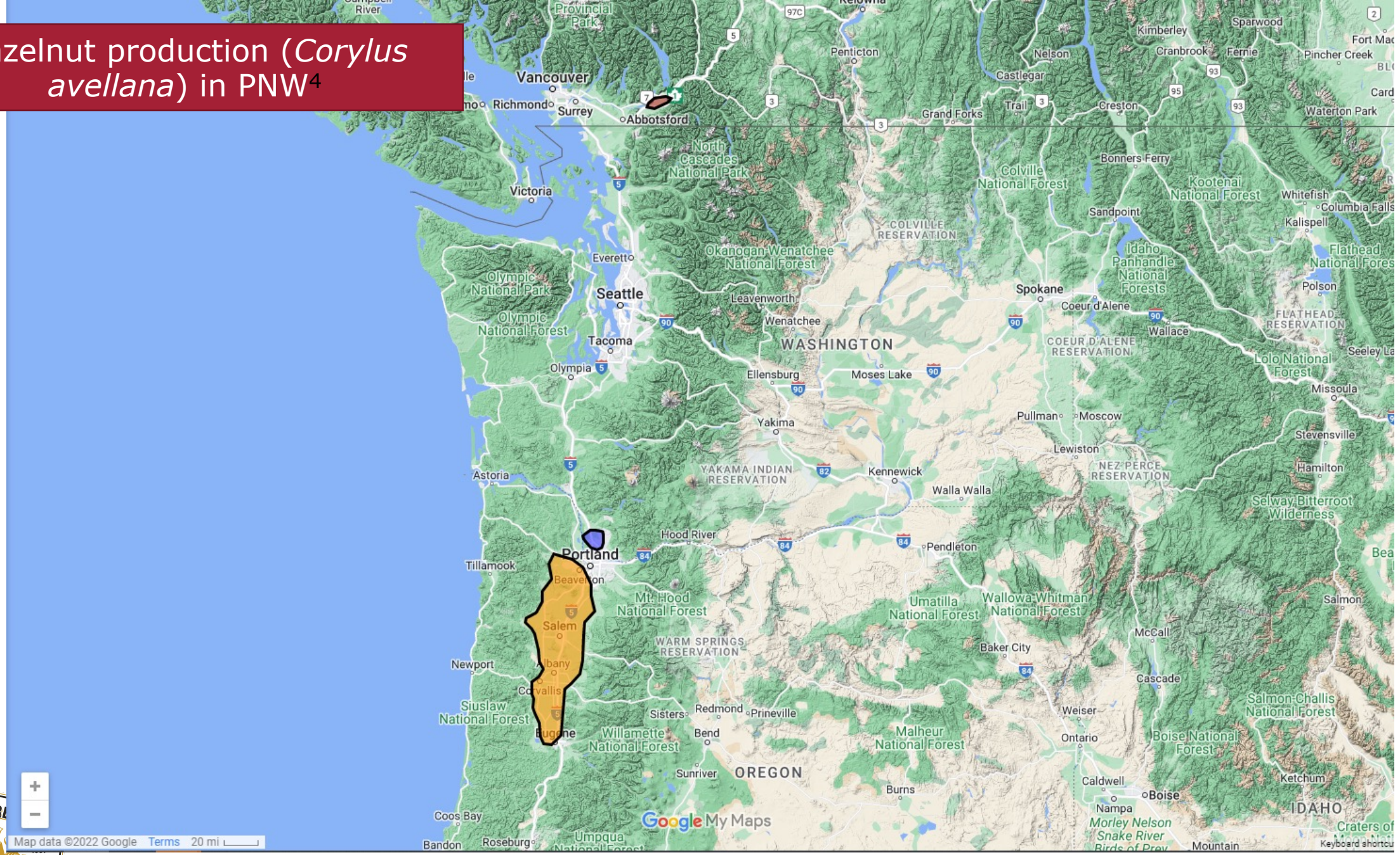
Hazelnuts (*Corylus avellana*)

- Most US production comes from Oregon
- Harvested in September
- Nuts fall to ground, then they are gathered
- Production has increased dramatically since 2010
- 77,500 tons produced in 2021 in Oregon

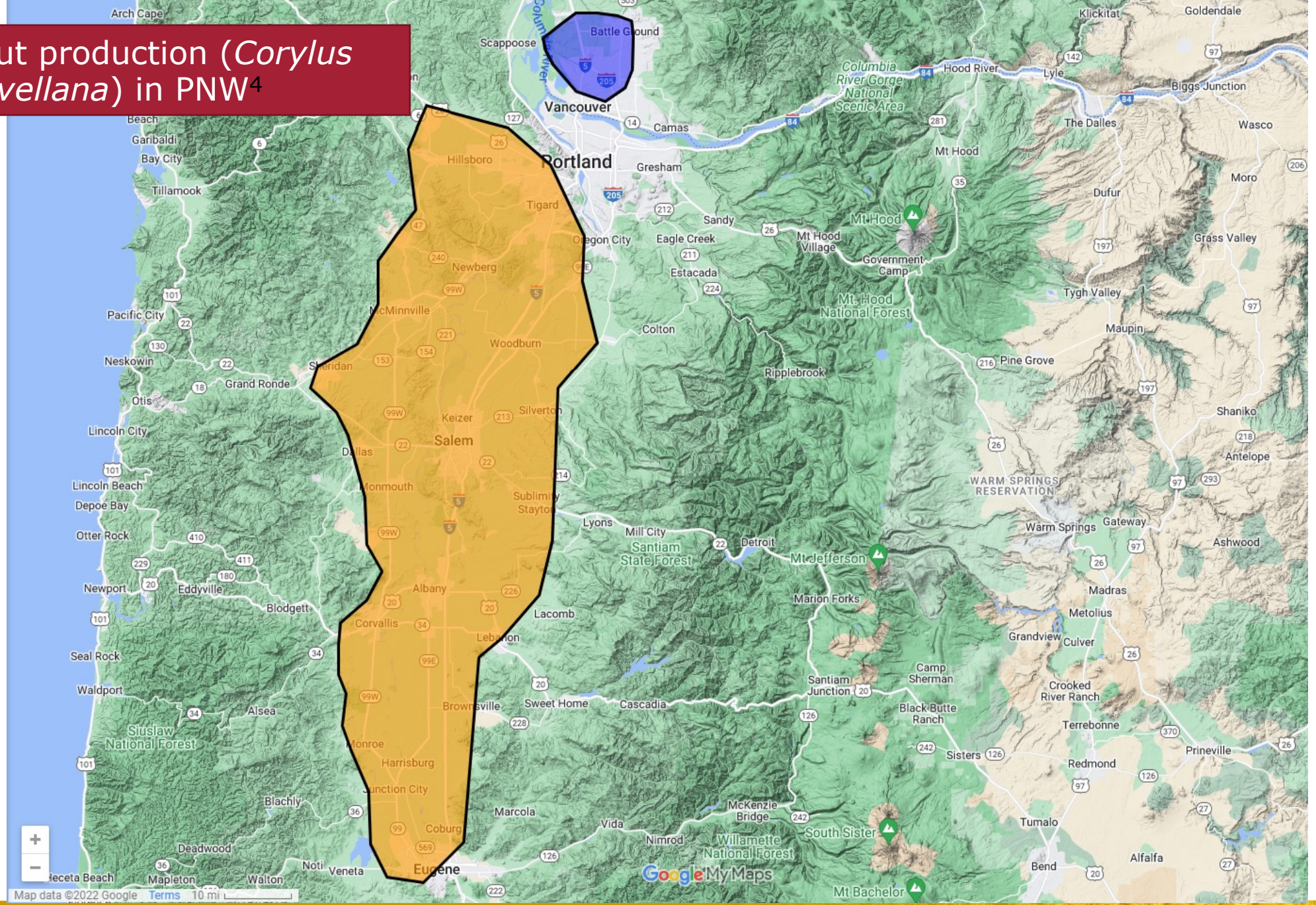
Hops (*Humulus lupulus*)

- Over 116 million pounds grown in PNW in 2021
- Harvested in late August - September
- Bines are cut, hops are removed and kilned, then [pelletized]
- 58,000 tons produced in 2021 in Oregon[3]

Hazelnut production (*Corylus avellana*) in PNW[4]



Hazelnut production (*Corylus avellana*) in PNW[4]



OREGON'S RECORD HIGH PRODUCTION: 1980–2020

Crop	Amount	Unit	Year
Apples	105,000	tons	1987
Blueberries	155,500,000	lbs	2019
Corn for grain	15,665,000	bu	**2020**
Corn for silage	1,161,000	tons	2013
Cranberries	604,000	bbls	**2020**
Grapes	77,000	tons	2017
Hay	3,891,000	tons	2016
Hazelnuts	63,000	tons	**2020**
Hops	13,782,400	lbs	1995
Onions	16,214,000	cwt	2007
Pears	258,000	tons	1994
Potatoes	30,683,000	cwt	2000
Ryegrass, annual	266,460,000	lbs	1999
Sweet cherries	66,000	tons	2009
Wheat	77,400,000	bu	1980

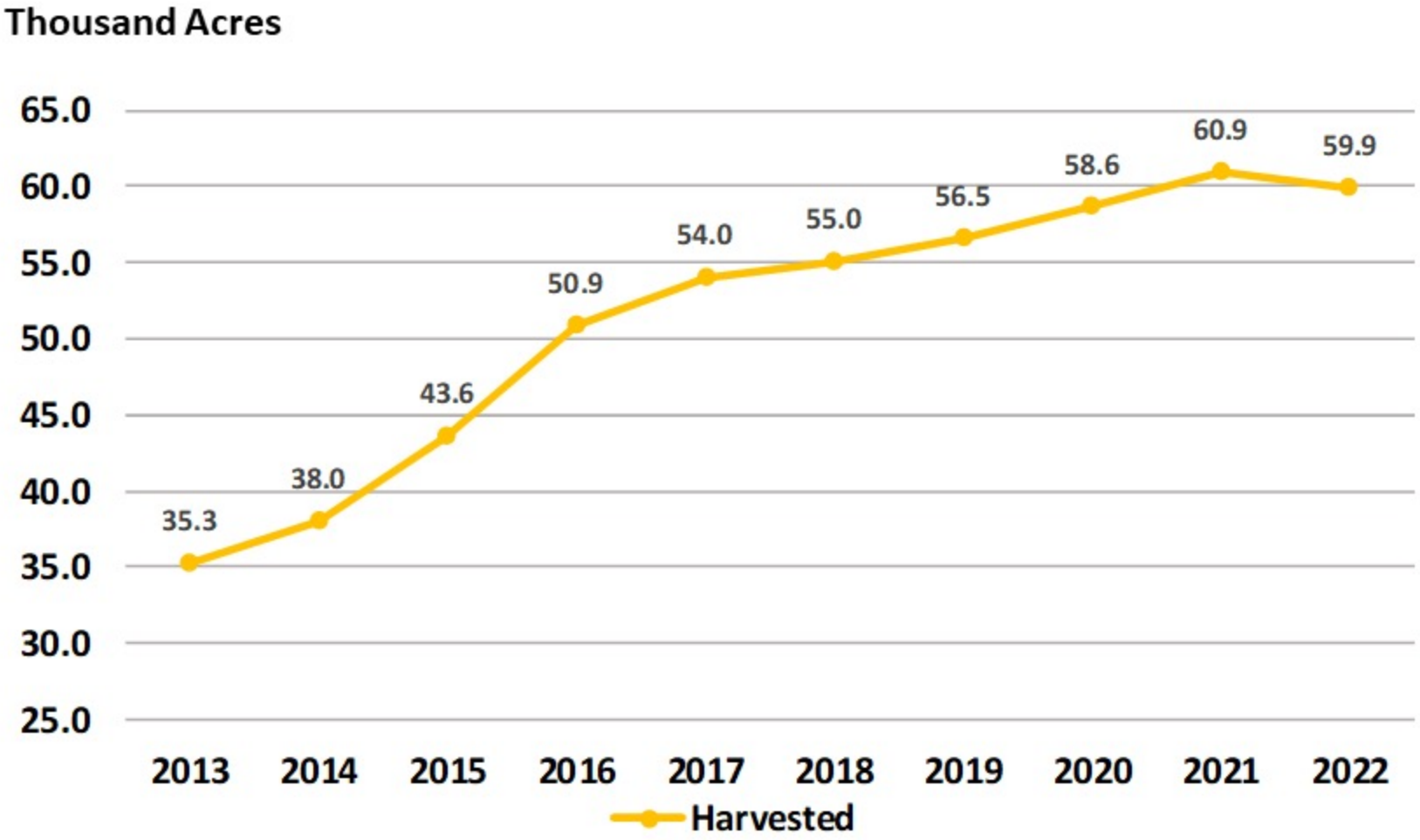
OREGON'S TOP 20 AGRICULTURAL COMMODITIES: 2020

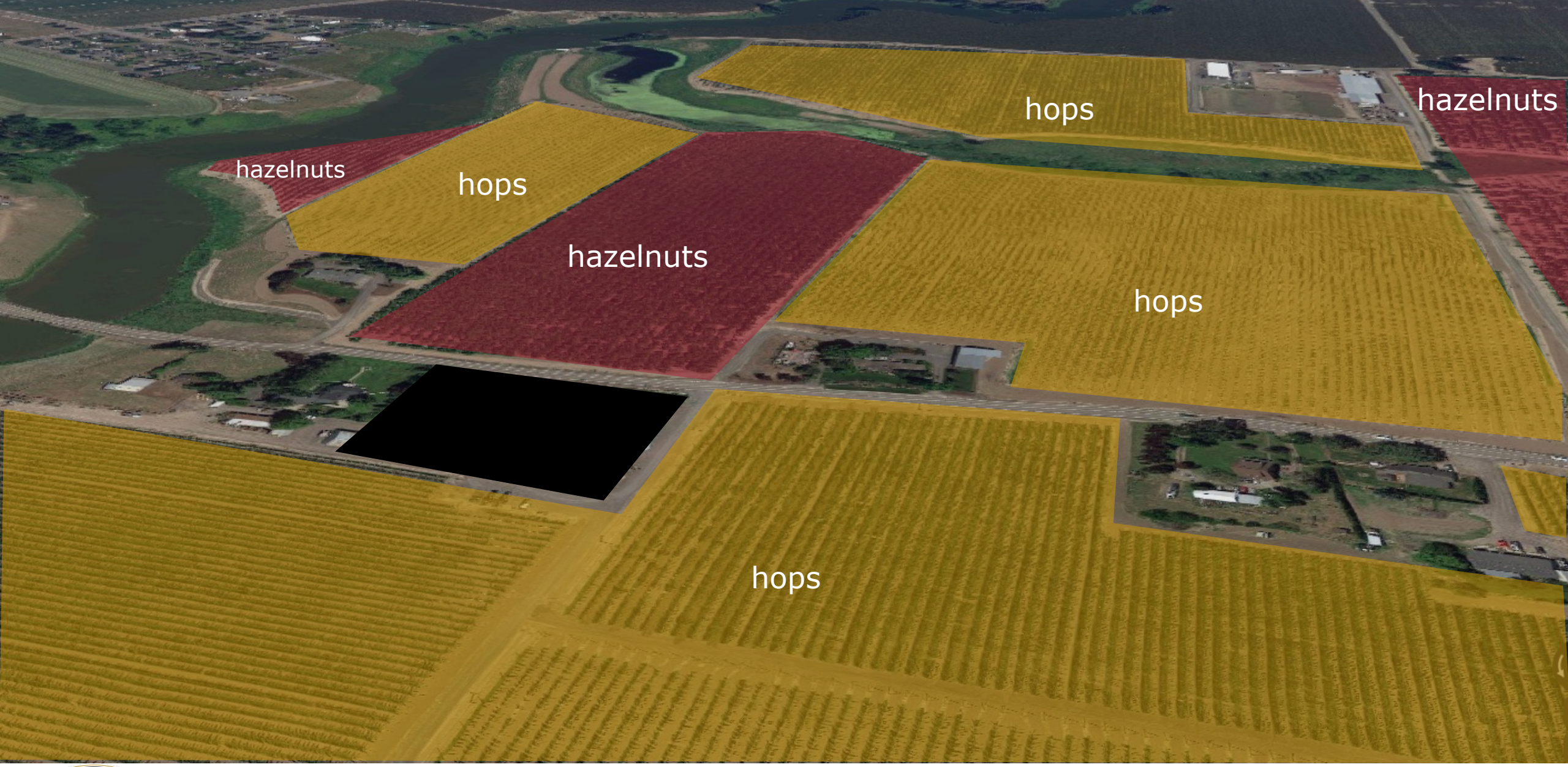
Rank	Commodity	Value of Production
1	Greenhouse & nursery[1]	$1,188,911,000
2	Cattle & calves	$587,848,000
3	Hay	$569,160,000
4	Milk	$557,348,000
5	Grass seed[2]	$458,367,000
6	Wheat	$273,760,000
7	Potatoes	$216,810,000
8	Grapes for wine[3]	$157,900,000
9	Cherries	$133,826,000
10	Hazelnuts	$132,300,000
11	Blueberries	$119,648,000
12	Onions	$118,665,000
13	Christmas trees	$106,912,000
14	Pears	$97,552,000
15	Corn, grain	$77,542,000
16	Hops	$74,812,000
17	Eggs	$72,999,000
18	Dungeness crab[4]	$72,643,709
19	Sweet corn	$41,034,000
20	Apples	$39,208,000

Hop Acres [6]

United States

Hazelnuts - ~87k





Google Maps

Google Maps

Google Maps

Google Maps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LH


9

Analytical Methods

For allergen detection in foods

Methods of detection

- ELISA
  - Most popular for allergen detection in foods
  - Robust
  - Detects protein
- PCR
  - Less popular for allergen detection
  - More technical
  - Detects DNA
- Lateral flow
- Mass spectrometry
  - Very technical / complex / expensive

Detection limit claims of commercial kits

Type	Name	LOD in ppm (from manufacturer)
ELISA	RIDASCREEN®FAST Hazelnut	0.19
ELISA	Eurofins SENSISpec Hazelnut	0.3
ELISA	Romer Labs AgraQuant® Hazelnut	0.3
ELISA	Crystal Chem Hazelnut ELISA Kit II	0.16
PCR	SureFood® ALLERGEN Hazelnut	0.4
PCR	Biotecon foodproof® Hazelnut	1
Lateral Flow	bioavid Lateral Flow Hazelnut	1
Lateral Flow	AllergenControl™ Hazelnut Residue Lateral Flow	1
Lateral Flow	Romer Labs AgraStrip® Hazelnut	5
Lateral Flow	Eurofins SENSIStrip Hazelnut	1?
Lateral Flow	Neogen Reveal® 3-D	5
Lateral Flow	Hygiena AllerTox	20
Lateral Flow	3M Hazelnut Protein Rapid Kit	2


Experimental Design

Method Comparison

ELSIA vs. PCR

RIDASCREEN®FAST Hazelnut (ELISA)

10

SureFood® ALLERGEN Hazelnut (PCR)

11

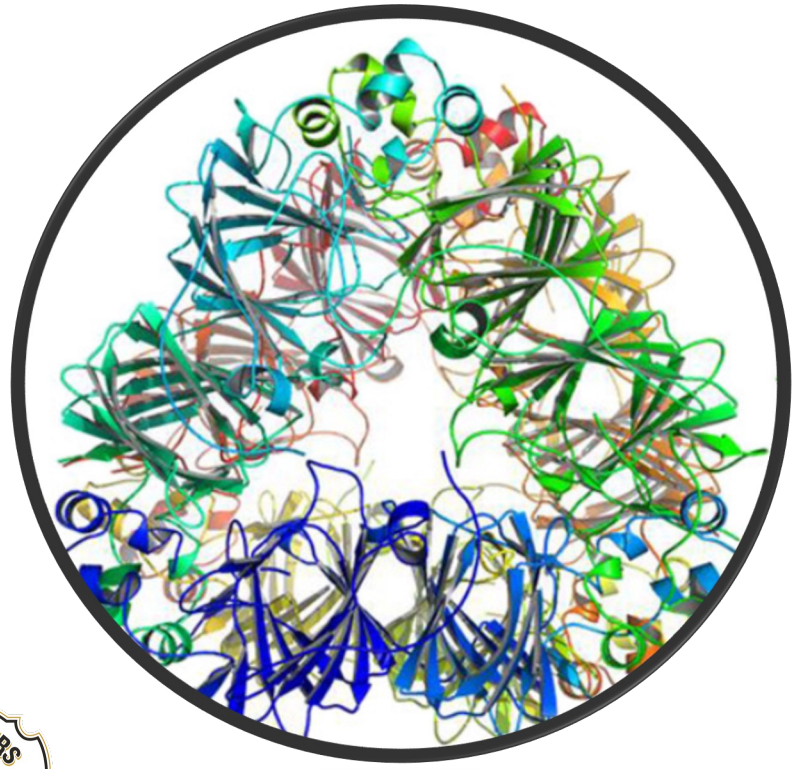
RIDASCREEN®FAST Hazelnut (ELISA)

SureFood® ALLERGEN Hazelnut (PCR)

Detection targets

RIDASCREEN®FAST Hazelnut (ELISA)

- Detects proteins from hazelnut that are allergenic
  - Example: Cor a 9 12



SureFood® ALLERGEN Hazelnut (PCR)

- Detects DNA of hazelnut (*Corylus avellena*)

13

Primers	Length (bp)	Sequence (5′ → 3′)
HazelnutITSdir	20	GAGACACTCGTGCCTTCTTG
HazelnutITSinv	17	GGAGCACTCTTTAGTTGAAGTTCC

Extraction strategies

RIDASCREEN®FAST Hazelnut (ELISA)

- Uses skim milk powder (**SMP**) as a blocking agent
  - Non-reacting substance used to prevent unspecific binding
- Uses allergen extraction buffer (**AEB**)
  - Most use a buffer solution containing DTT (dithiothreitol) and/or phenol to separate and protect proteins from food matrices
- Extraction occurs at 60°C with occasional shaking, and then solids are centrifuged away. **Extracted proteins** remain in the supernatant.

SureFood® ALLERGEN Hazelnut (PCR)

- Uses **lysis buffer, ethanol,** and **Proteinase K** to release DNA from cells.
- Extraction occurs at 65°C with occasional shaking.
- Spin filters and wash buffer separate DNA from the matrix.
- **Purified nucleic acids (DNA)** are eluted from the spin filter in the final step.

Extraction method comparison

RIDASCREEN®FAST Hazelnut (ELISA) 10

1. 1g sample
2. Sample prep with skim milk powder (SMP)
3. Extraction at 60°C
4. Filtration or centrifugation
5. Second filtration or centrifugation (optional)

- Hazelnut **proteins** will be in supernatant.
- Takes about 20 minutes

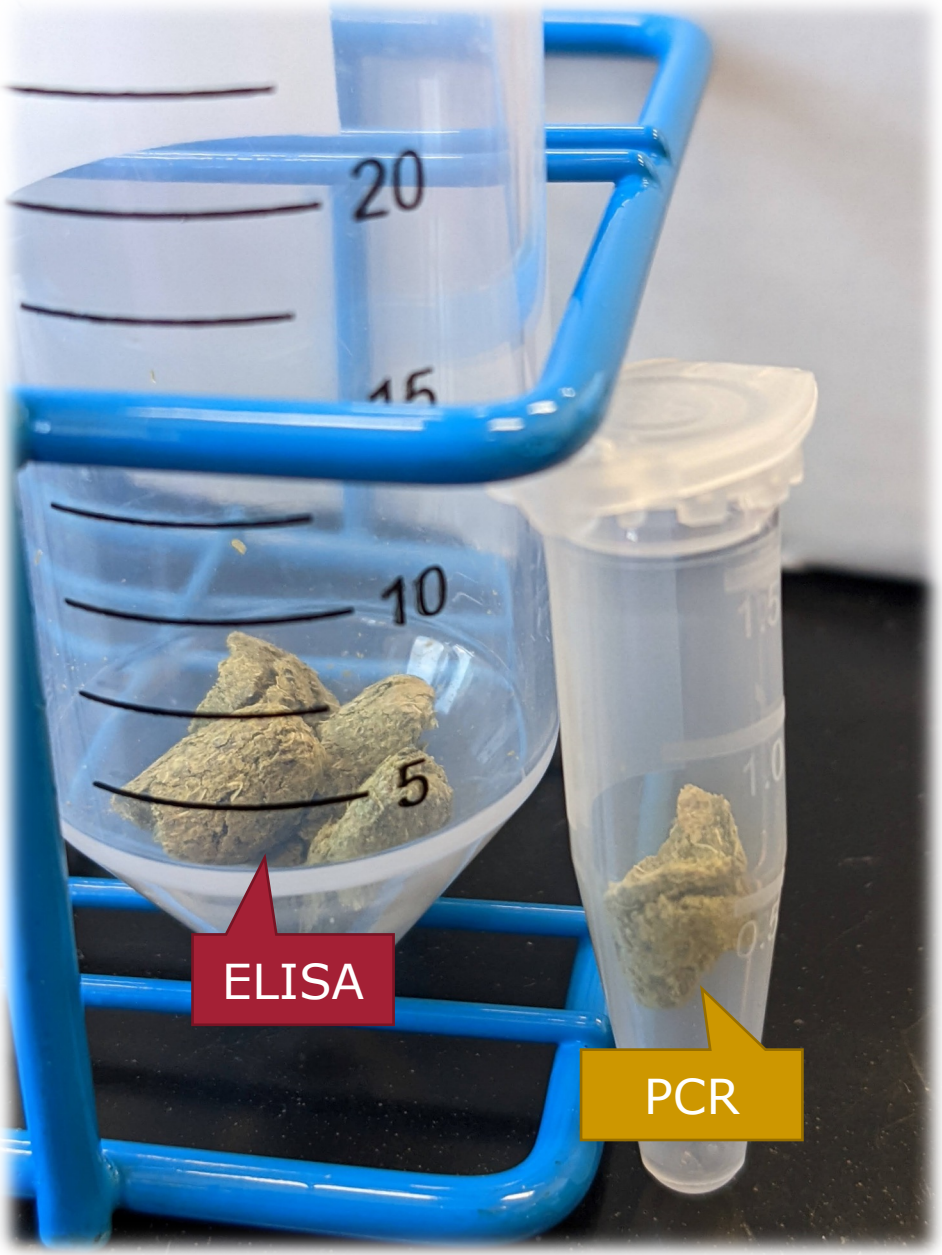
SureFood® ALLERGEN Hazelnut (PCR) 11

1. 100mg sample
2. Sample prep
3. Lysis
4. Pre-filtration
5. Binding of **nucleic acids**
6. Purification and drying
7. Elution of **nucleic acids**
8. Second purification (optional)

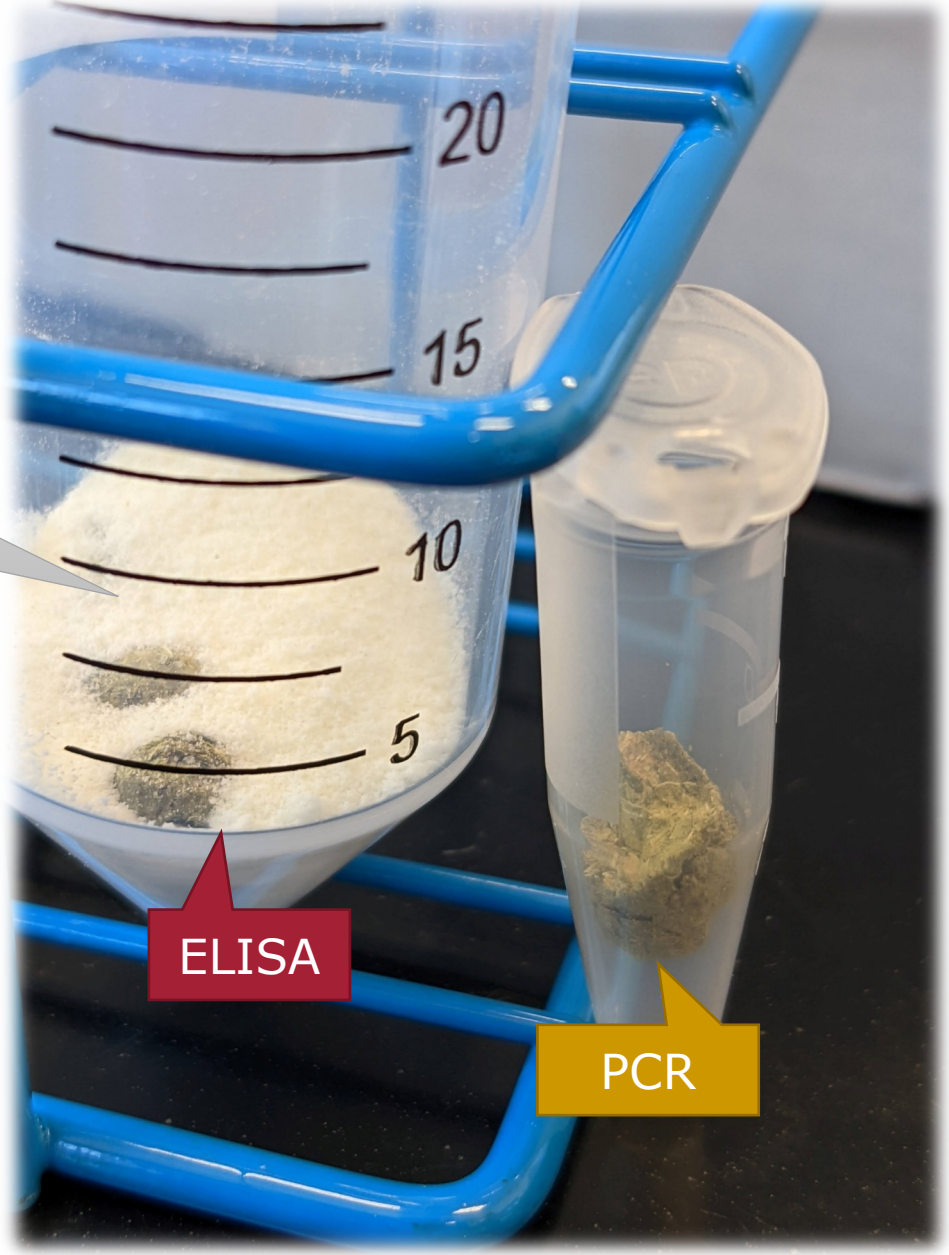
- Takes about 40 minutes

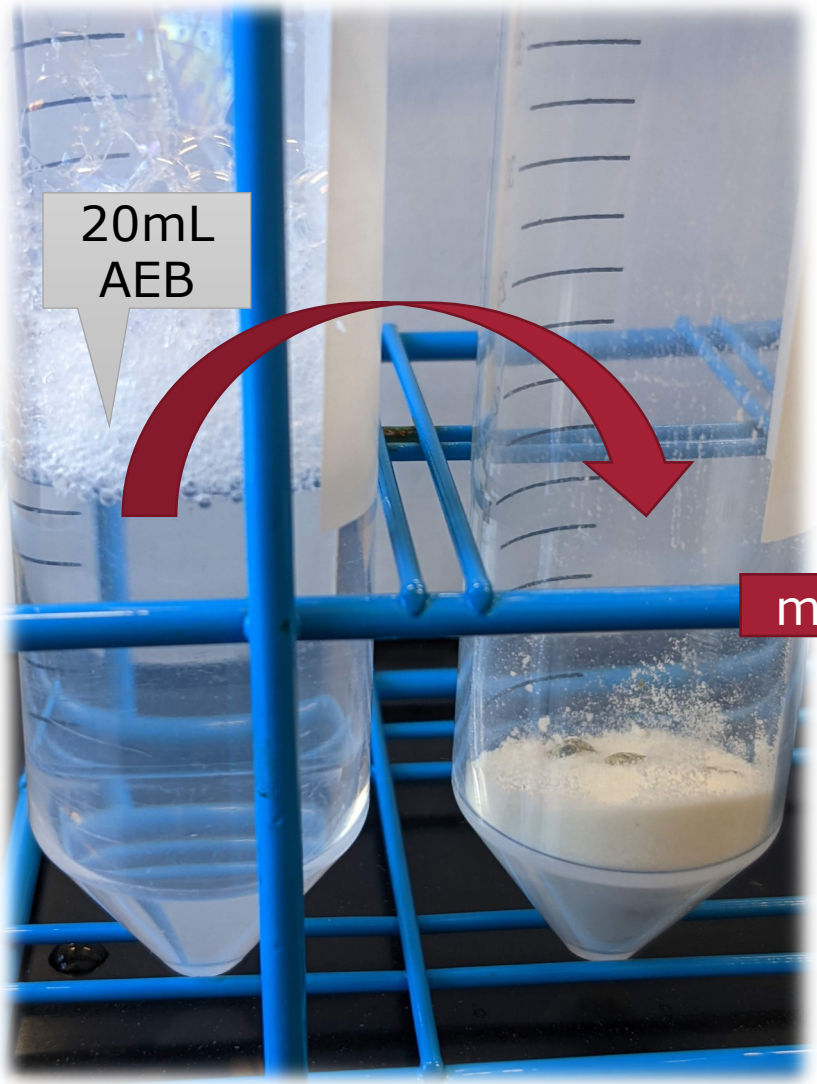
RIDASCREEN®FAST Hazelnut (ELISA)

SureFood® ALLERGEN Hazelnut (PCR)

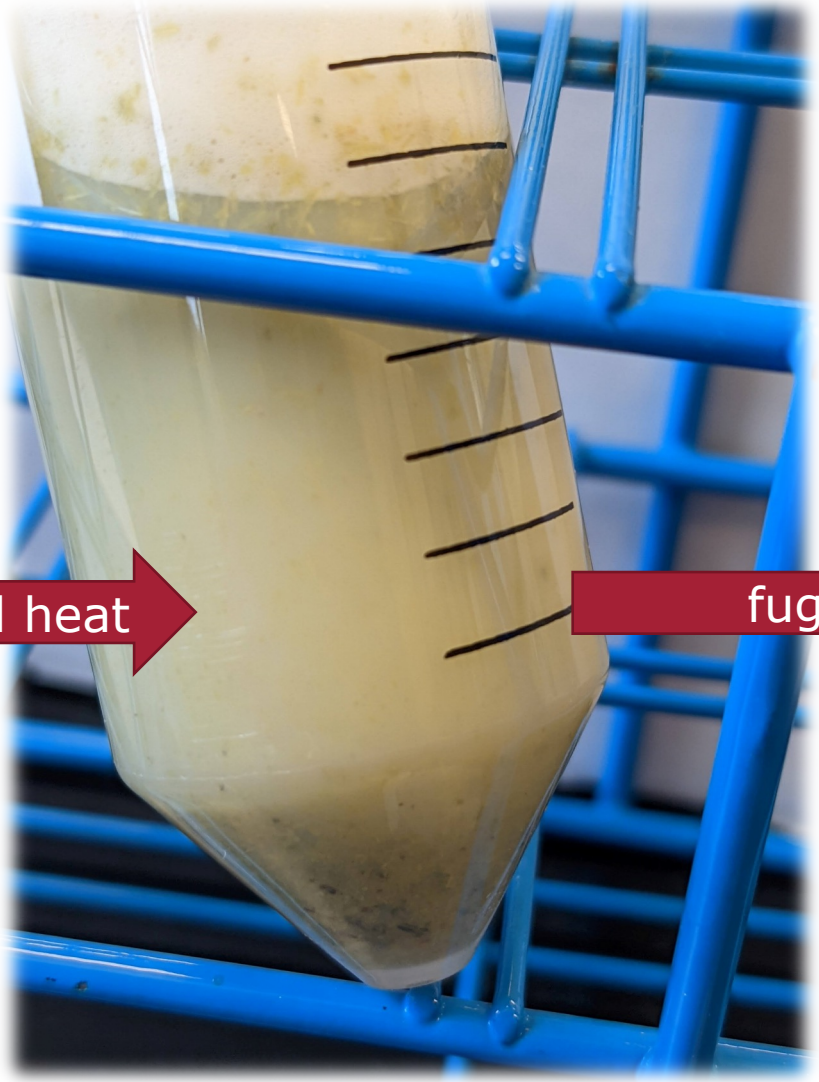


1.0g
SMP





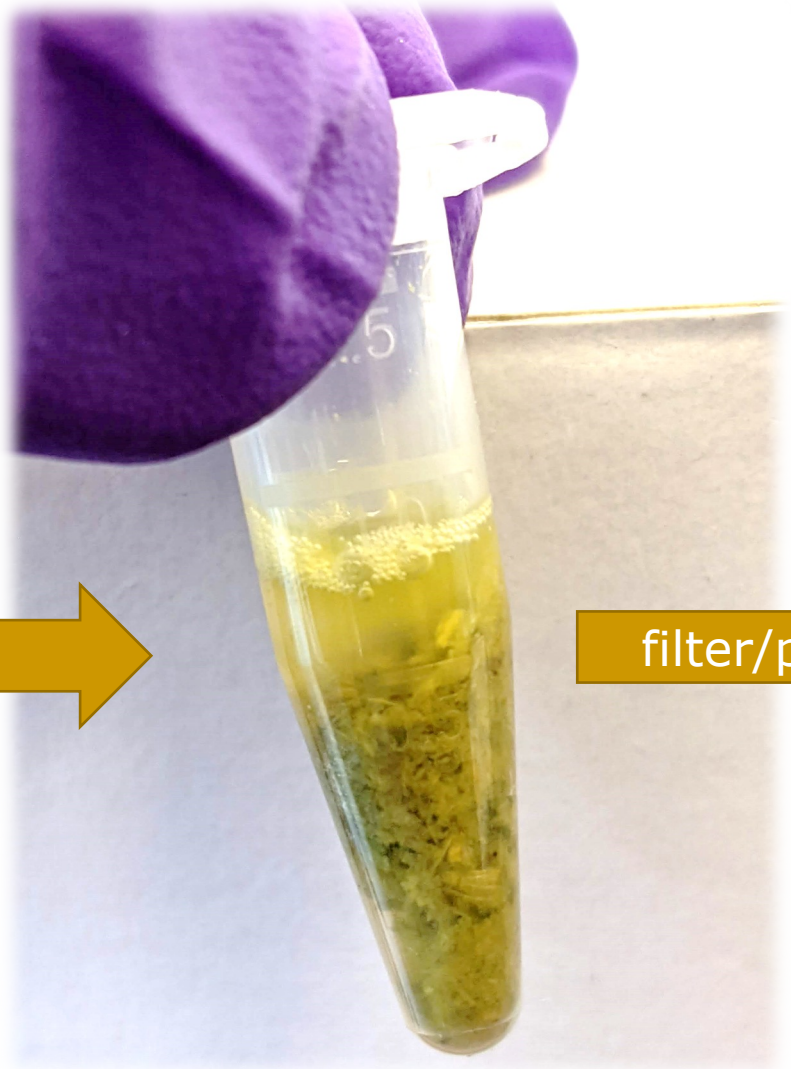
mix and heat



fuge

Protein for ELISA

lysis



filter/purify

DNA for PCR

Test method comparison

RIDASCREEN®FAST Hazelnut (ELISA)

1. Use extraction with **protein**
2. Pipette 100µL of extraction into antibody-coated wells
    1. Incubate for 10 minutes at room temp
    2. Wash 3x
3. Pipette 100µL of conjugate into each well
    1. Incubate for 10 minutes at room temp
    2. Wash 3x
4. Pipette 100µL of substrate/chromogen into each well
    1. Incubate for 10 minutes in the dark
    2. Add stop solution
5. Read absorbance at 450nm

SureFood® ALLERGEN Hazelnut (PCR)

1. Use extracted **nucleic acids**
2. Prepare master mix for PCR
3. Add 5µL of extraction to 20µL of master mix
4. Run PCR

Three different experiments

First trial with commercial kits:

- RIDASCREEN®FAST Hazelnut (ELISA)
- SureFood® ALLERGEN Hazelnut (PCR)

ELISA vs. PCR

Second trial with whole cone hops

- Positive control spike with hazelnut dust

Third trial with pelletized hops

- With positive control

14

18

Types of samples

17

- Hops
- Chocolate
- Beer
- Nuts

15

IMPERIAL STOUT WITH CHOCOLATE AND HAZELNUT COFFEE AGED IN OAK BOURBON BARRELS

"The perfectly balanced and decadent drinking experience of KBS meets the classic taste of hazelnut. Taking this bourbon barrel-aged stout to a new level, KBS Hazelnut allows the existing premium coffee and chocolate notes to soar to new heights when accented by the nutty sweetness of hazelnut."

Trial 1

Sample list (for trial 1)

1st test

1. Whole leaf hops
2. Pure hazelnut dust
3. Dark chocolate with hazelnuts
4. KBS Hazelnut
5. KBS Hazelnut + hazelnut dust spike
6. Whole leaf hops + hazelnut dust spike

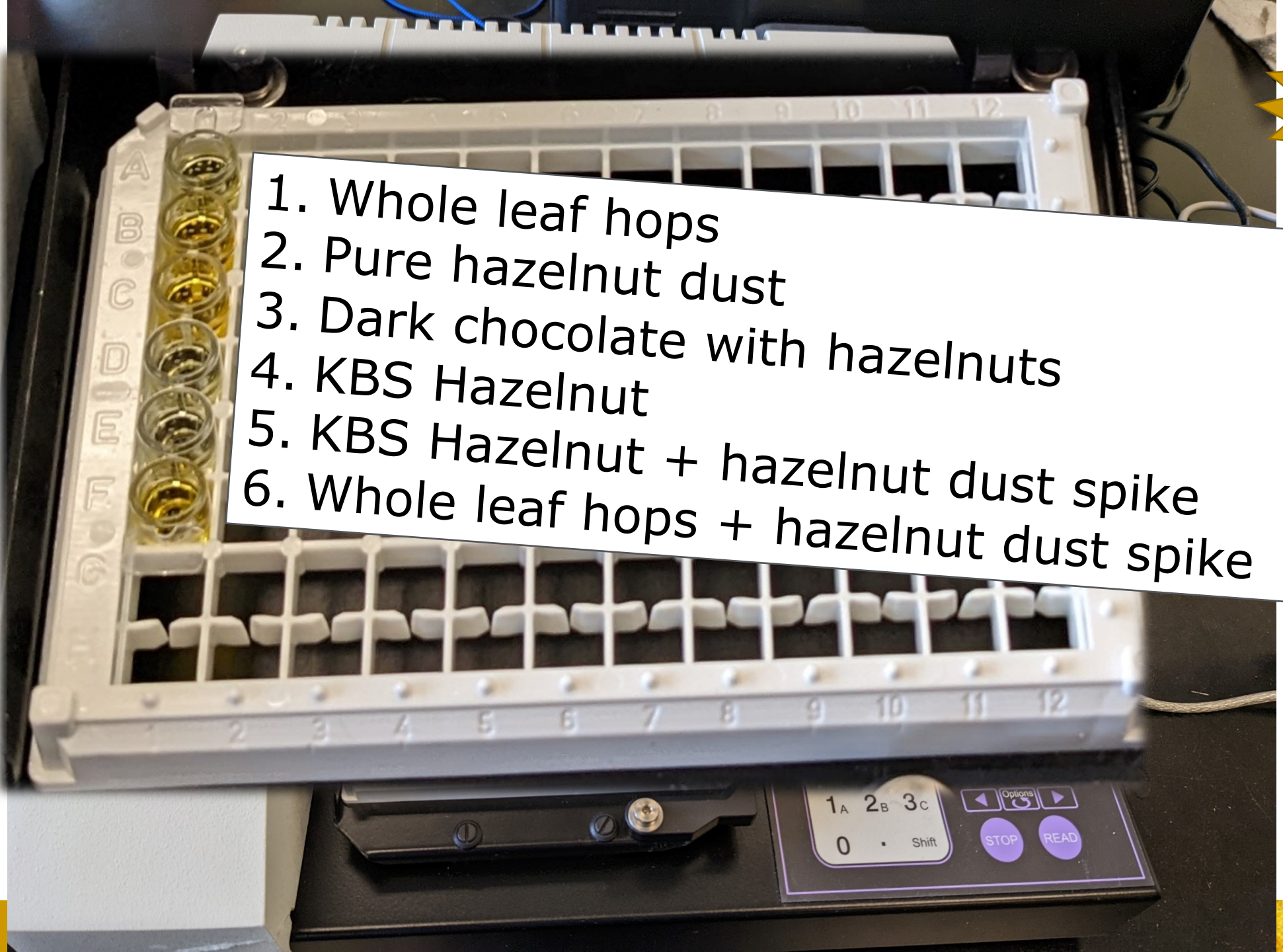
Image from PCR extraction

Results from 1st Test

Trial 1 results

1st test

Number	Name	ELISA mg/kg hazelnut	PCR results
1	Whole leaf hops	< 2.50	Negative
2	Pure hazelnut dust	> 20.00	Positive (Cp 17.23)
3	Dark chocolate with hazelnuts	> 20.00	Positive (Cp 21.12)
4	KBS Hazelnut	< 2.50	Negative
5	KBS Hazelnut + hazelnut dust spike	< 2.50	Positive (Cp 33.32)
6	Whole leaf hops + hazelnut dust spike	> 20.00	Positive (Cp 25.06)


1. Whole leaf hops
2. Pure hazelnut dust
3. Dark chocolate with hazelnuts
4. KBS Hazelnut
5. KBS Hazelnut + hazelnut dust spike
6. Whole leaf hops + hazelnut dust spike
1st test
STOP
READ
Options
Shift

FAST Hazelnut
29. Mar. 2022, 10:56:30, Spline, 450 nm, Ser.No: 0, Version: 1.1.1

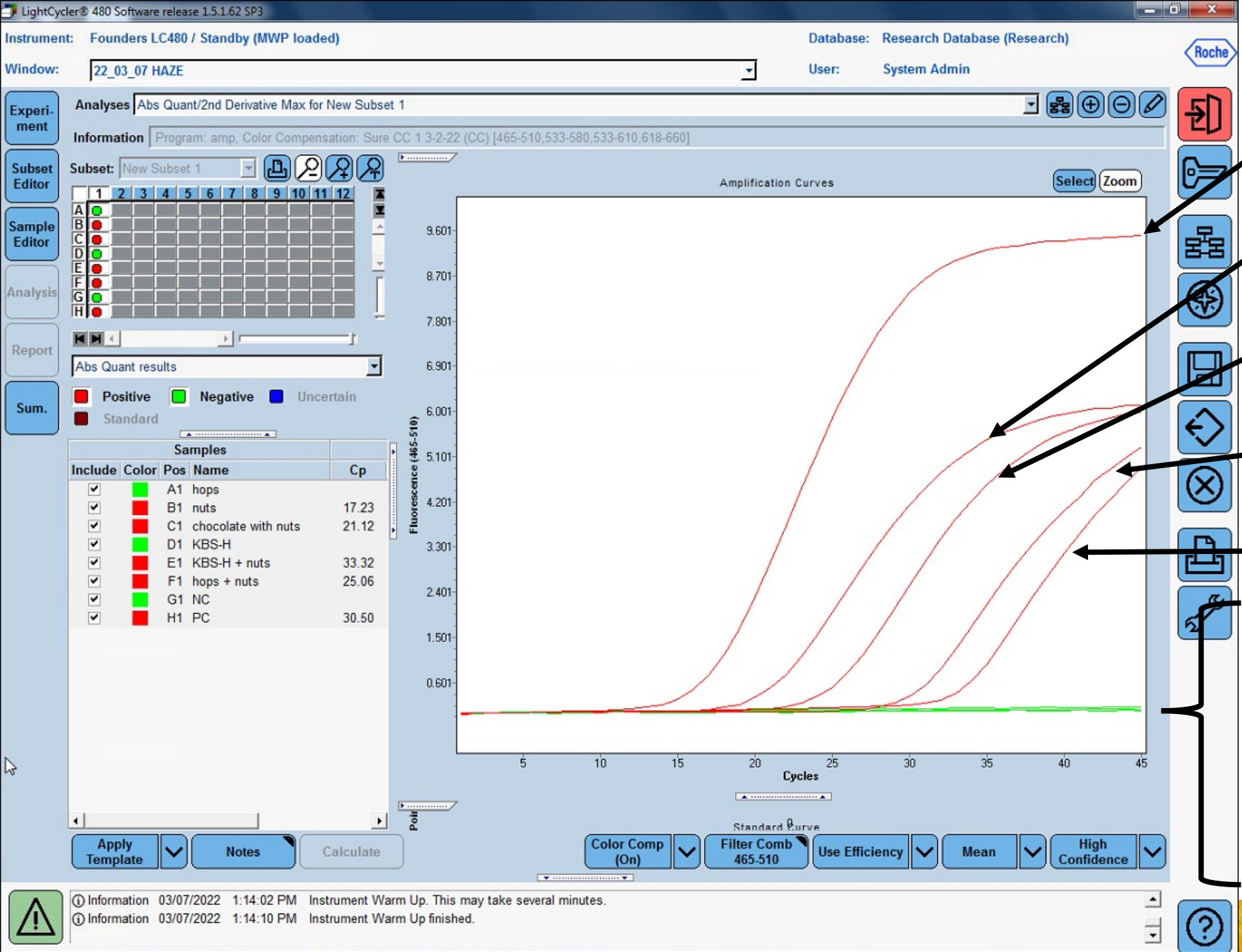
1st test

Standards

Ser. No.	Concentration mg/kg	Absorbance (Mean)	(CV)	B/Bmax (%)
1	0.00	0.120	8.2	6.6
2	2.50	0.678	4.7	37.6
3	5.00	1.017	9.7	56.3
4	10.00	1.400	2.9	77.6
5	20.00	1.805	3.7	100.0

Samples

Ser. No.	ID	Absorbance (Mean)	(CV)	(%)	calculated mg/kg	* Factor	=	mg/kg hazelnut	mg/kg hazelnut
1.	Whole leaf hops	0.353E	0.0	19.6	< 2.50	1.00		< 2.50	
2.	Pure hazelnut dust	3.354E	0.0	185.8	> 20.00	1.00		> 20.00	
3.	Dark chocolate with hazelnuts	3.434E	0.0	190.2	> 20.00	1.00		> 20.00	
4.	KBS Hazelnut	0.246E	0.0	13.6	< 2.50	1.00		< 2.50	
5.	KBS Hazelnut + hazelnut dust spike	0.224E	0.0	12.4	< 2.50	1.00		< 2.50	
6.	Whole leaf hops + hazelnut dust spike	3.409E	0.0	188.9	> 20.00	1.00		> 20.00	



1st test

2. Pure hazelnut

3. Dark chocolate with hazelnuts

6. Hops with hazelnut dust

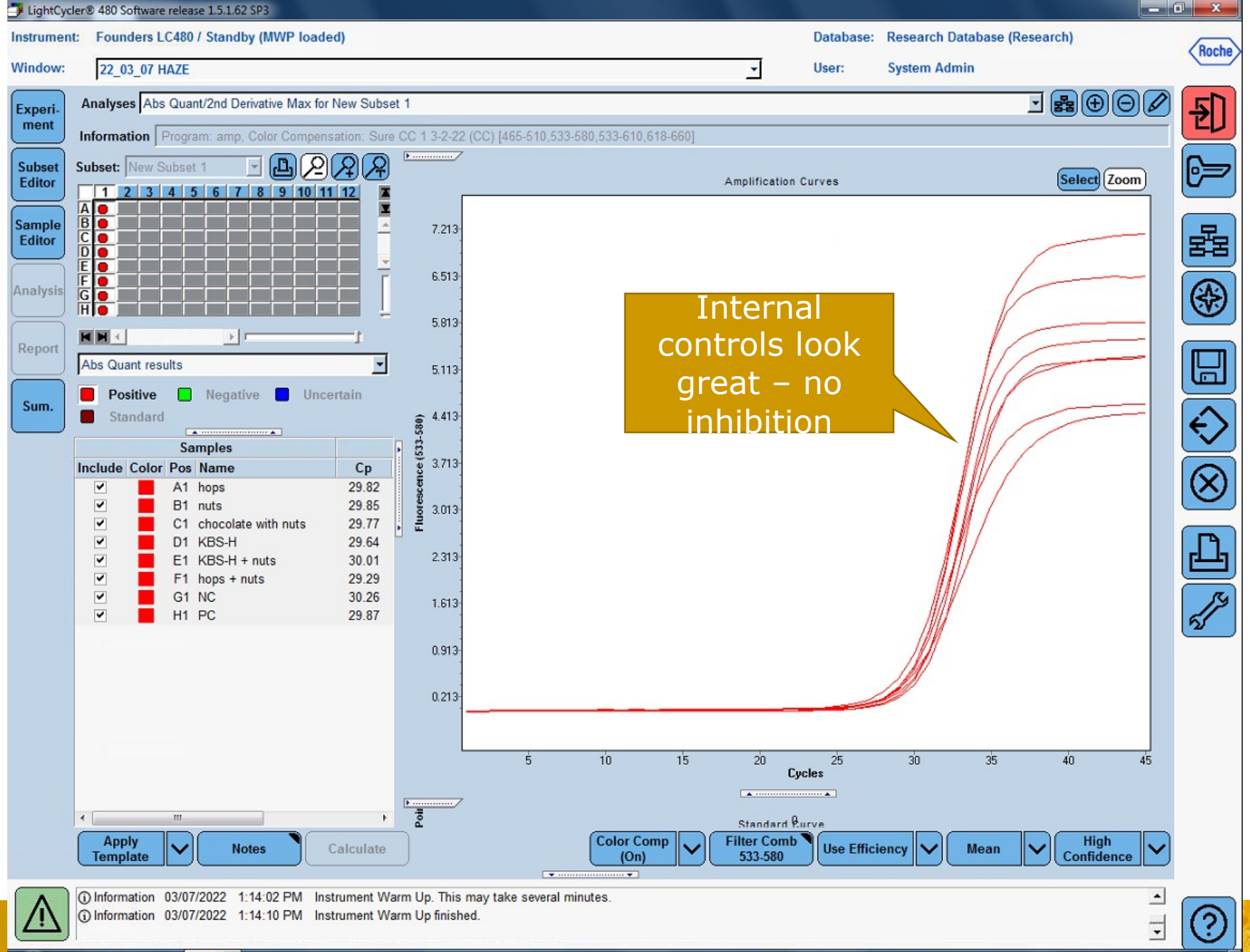
PC

5. KBS-H with hazelnut dust

1. Hops

4. KBS-H (straight)

NC



1st test

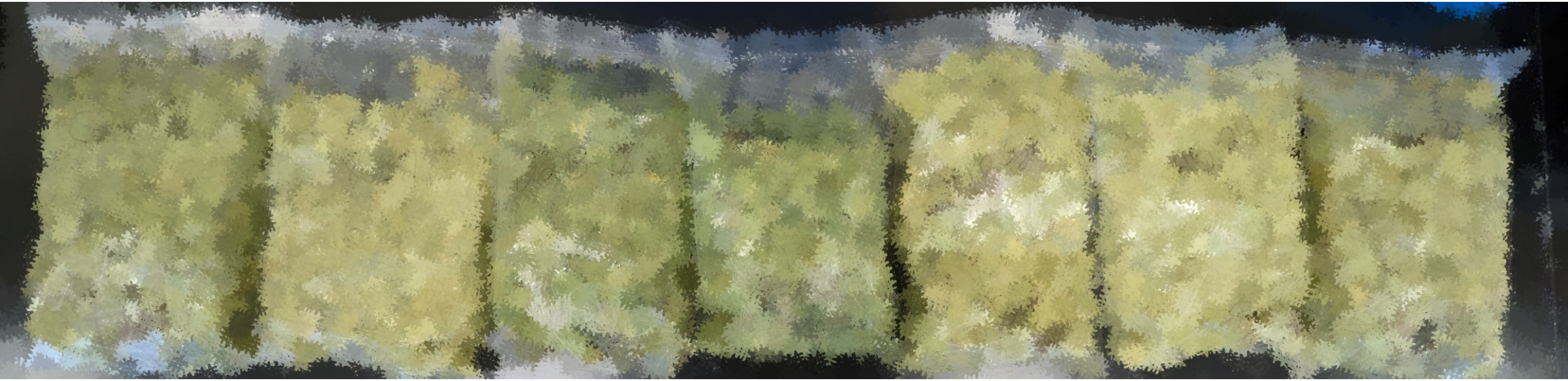
MASTER BREWERS ASSOCIATION OF THE AMERICAS EST 1887


Trial 2
more hops

Sample list (for trial 2)

2nd test

1. Whole leaf #1
2. Whole leaf #2
3. Whole leaf #3
4. Whole leaf #4
5. Whole leaf #5
6. Whole leaf #6
7. Whole leaf #7
8. Whole leaf #7 with hazelnut dust

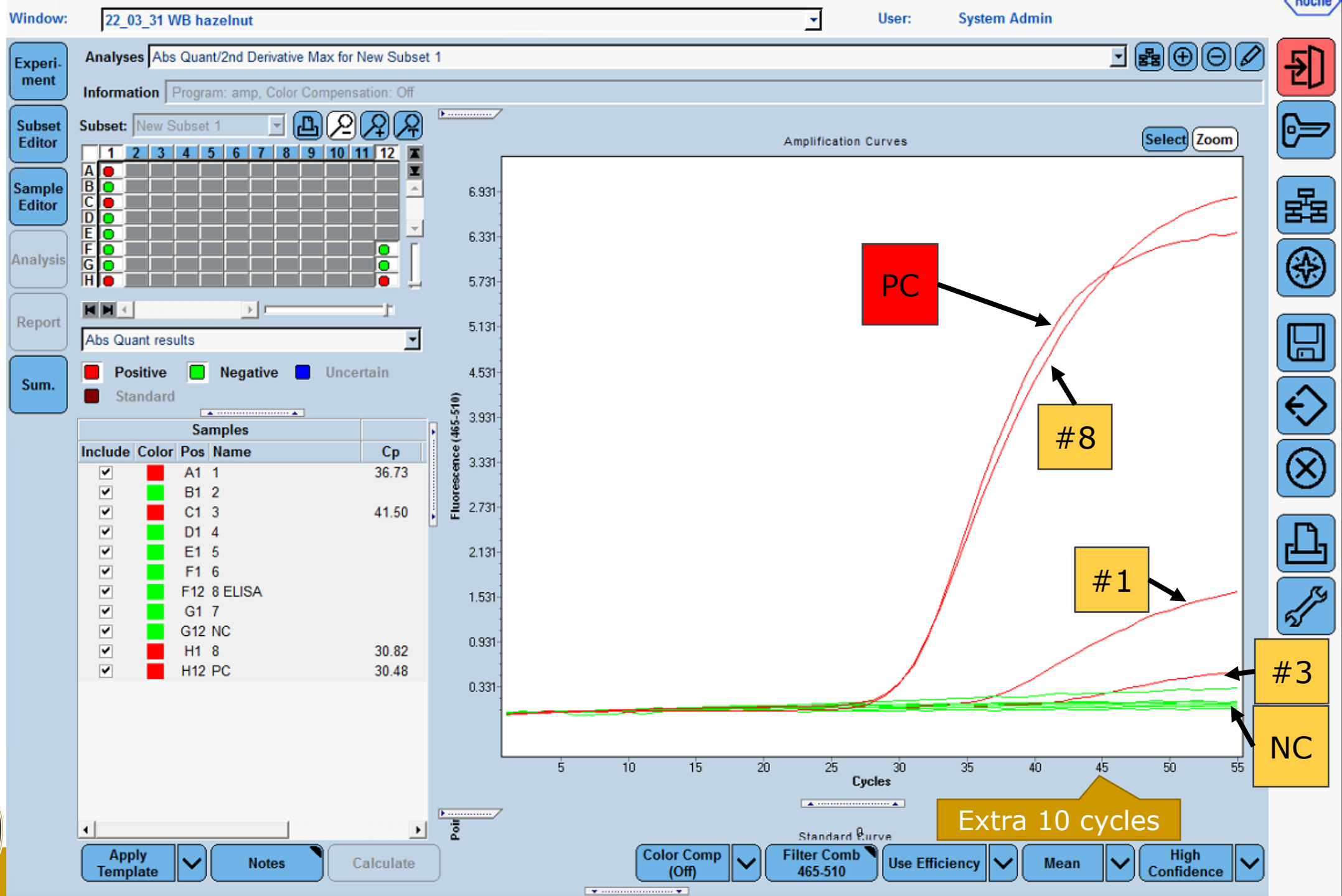


Results from 2nd Test

Trial 2 results

2nd test

Number	Name	mg/kg hazelnut	PCR results
1	Whole leaf #1	< 2.50	Weak positive (Cp 36.73)
2	Whole leaf #2	< 2.50	Negative
3	Whole leaf #3	< 2.50	Weak positive (Cp 41.50)
4	Whole leaf #4	< 2.50	Negative
5	Whole leaf #5	< 2.50	Negative
6	Whole leaf #6	< 2.50	Negative
7	Whole leaf #7	< 2.50	Negative
8	Whole leaf #7 + hazelnut dust	> 20.00	Positive (Cp 30.82)


Window:
22_03_31 WB hazelnut
User:
System Admin
Experiment
Subset Editor
Sample Editor
Analysis
Report
Sum.
Analyses
Abs Quant/2nd Derivative Max for New Subset 1
Information
Program: amp, Color Compensation: Off
Subset:
New Subset 1
Abs Quant results
Positive
Negative
Uncertain
Standard
Samples
Include Color Pos Name Cp
A1 1 36.73
B1 2
C1 3 41.50
D1 4
E1 5
F1 6
F12 8 ELISA
G1 7
G12 NC
H1 8 30.82
H12 PC 30.48
Amplification Curves
Select
Zoom
Fluorescence (465-510)
Cycles
PC
#8
#1
#3
NC
Extra 10 cycles
Apply Template
Notes
Calculate
Color Comp (Off)
Filter Comb 465-510
Use Efficiency
Mean
High Confidence

Trial 3

pellets

Sample list (for trial 3)

3rd test

1. Pelletized hops
2. Pelletized hops + hazelnut dust

16

17

Trial 3 results

3rd test

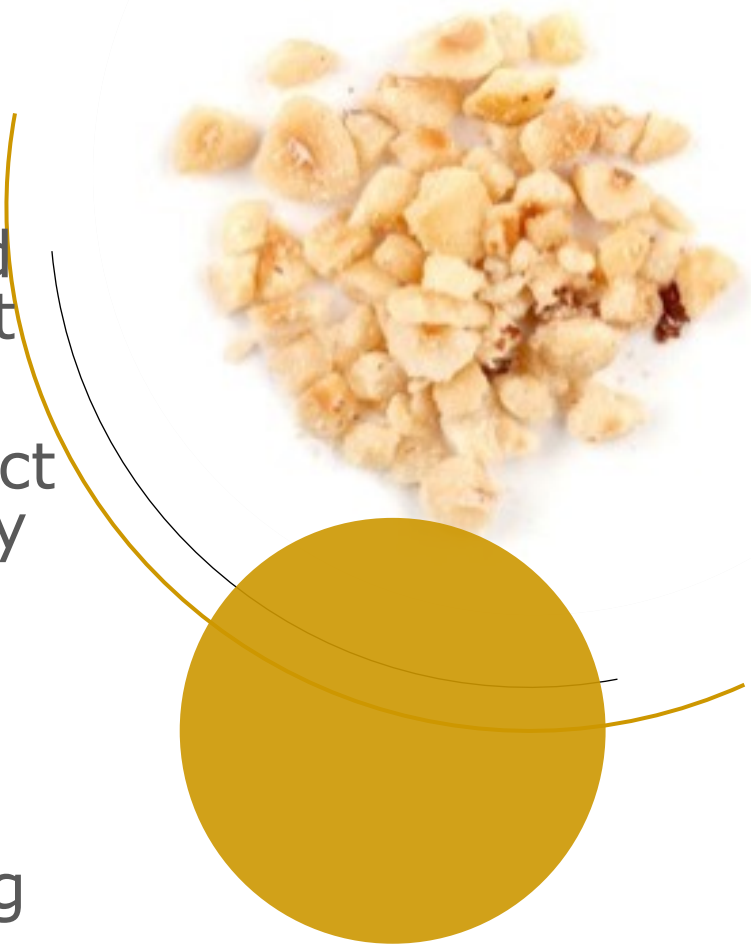
Number	Name	mg/kg hazelnut	PCR results
1	Pelletized hops	< 2.50	Negative
2	Pelletized hops + hazelnut dust	> 20.00	Positive (Cp 26.52)

16

17

Conclusions

Takeaway messages



- Allergens are an important food safety issue, and producers of ingredients and finished goods must be vigilant.
- ELISA and PCR test methods can effectively detect tree nut allergens in hops and dark beer, but they are difficult matrices to analyze.
- All 3rd party assays should be validated with a challenge study using hops before reporting results.
- In this study, PCR was more effective in detecting trace levels of hazelnut in hops and dark beer.
- More investigation into hop processing and potential cross-contamination is needed.

Future opportunities

- Environmental swabs during harvest / processing
- Brewing trials
  - Are proteins denatured? What about DNA?
- Quantification is possible
- Field testing
- Labeling??
- Other methods (lateral flow / MS)

Thank you

Hop Growers and Processors of PNW

Resources

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Q&A