Emerging Trends in Agricultural Diagnostics

NAGC

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National Agricultural Genotyping Center

Fargo, North Dakota

Talk Outline

Genetic Research Background

- Reproductive biology - lizard
- Conservation genetics – bird

Diagnostic Biotechnology

- qPCR

Current NAGC research

- Pathogens – honey bees
- Pathogens – row crops
- Traits – weeds

Undergraduate Interest - Reptiles

Masters Research

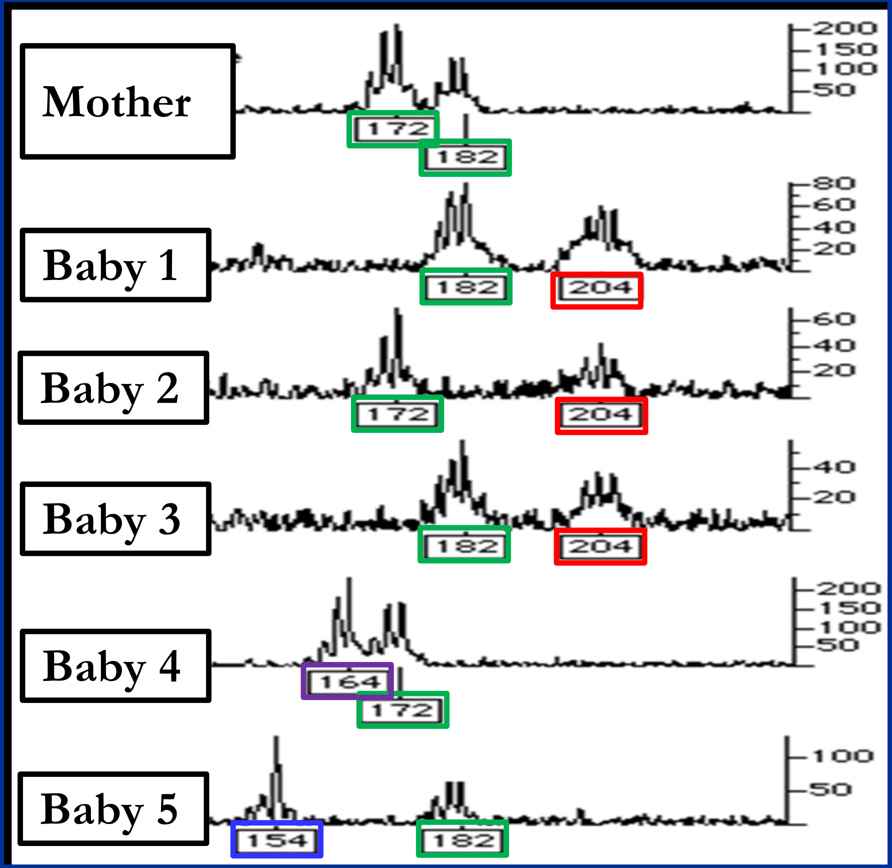
Do females have genetically diverse clutches?

Masters Research

Do females have genetically diverse clutches?

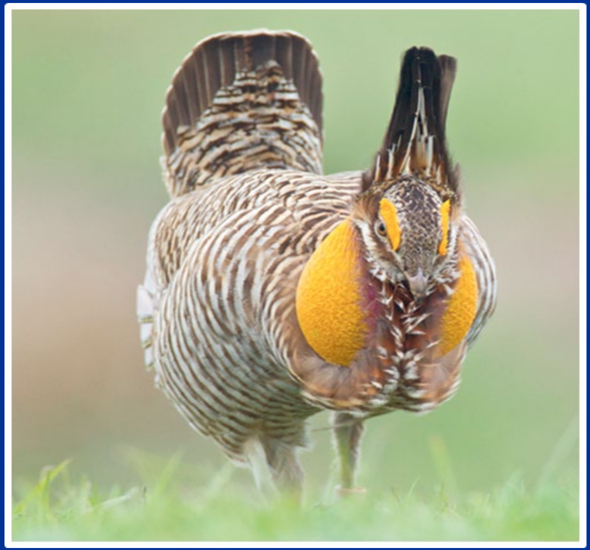
Yes!

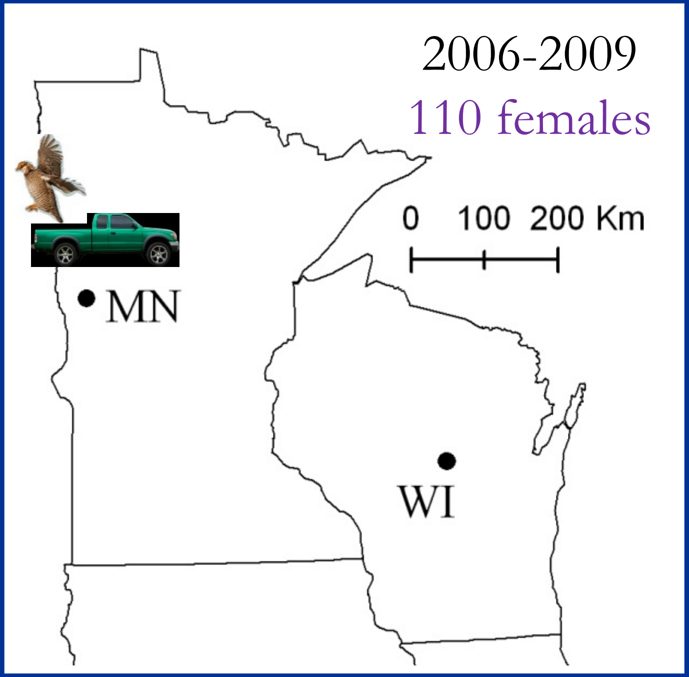
Paternity Analysis (1 locus)



Ph.D. Research

Can translocated birds boost genetic diversity in an endangered population?





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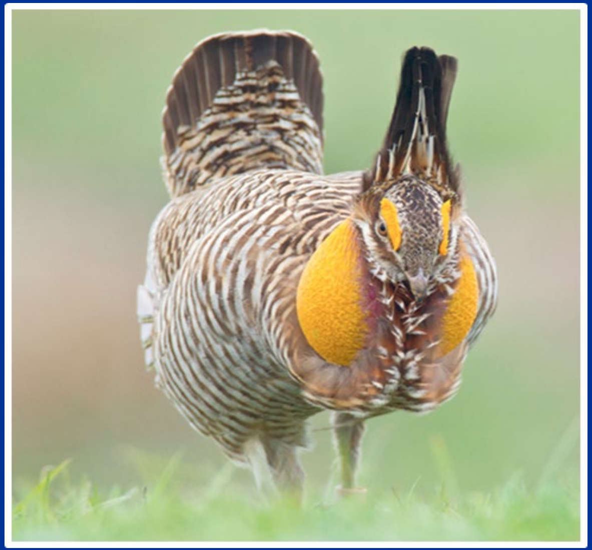
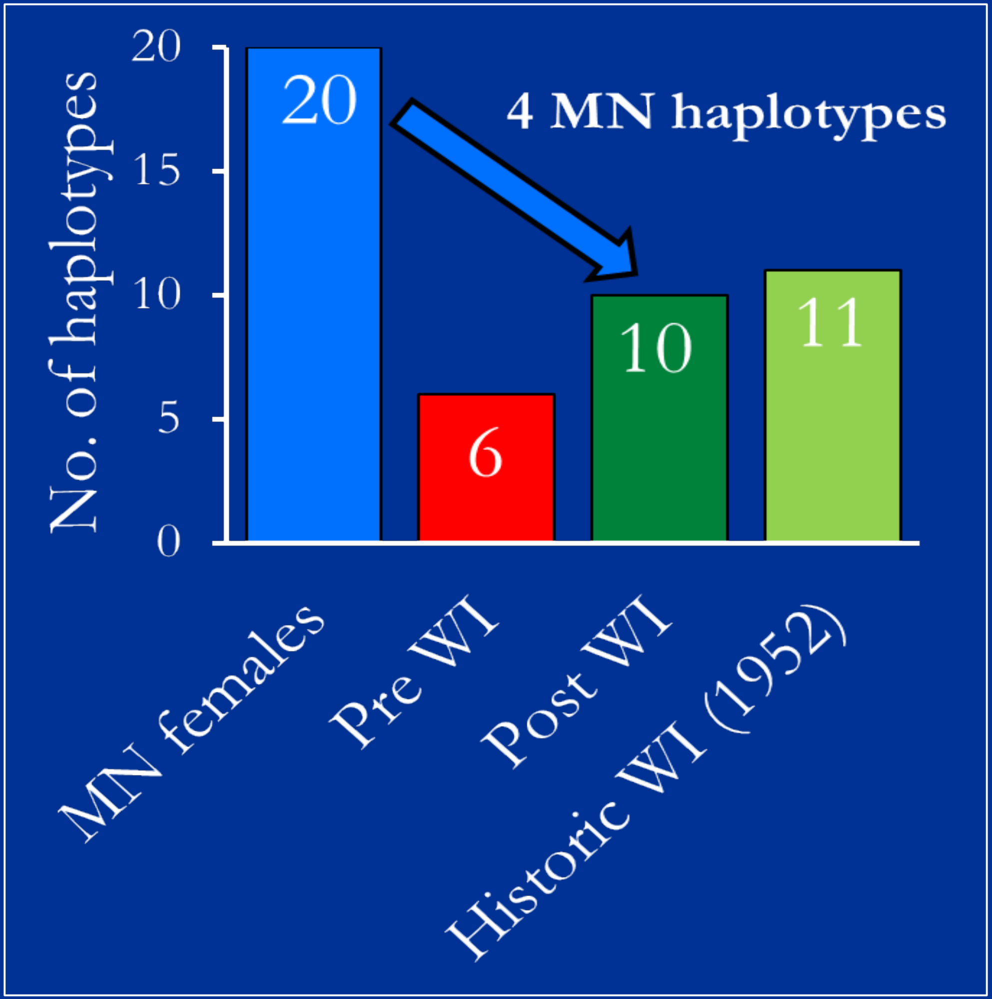
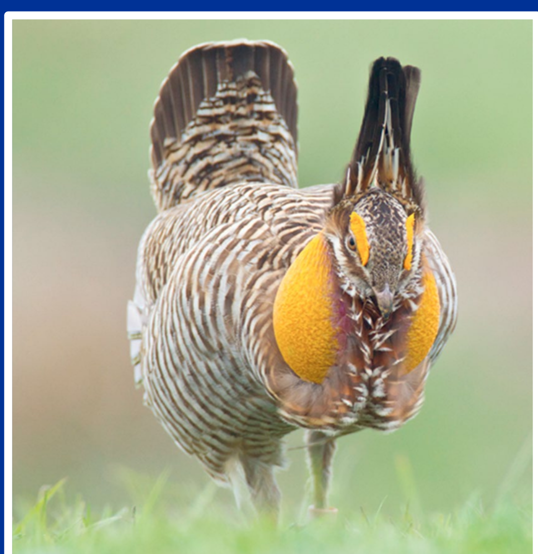




mtDNA diversity restored to near historic levels

Wildlife to Agriculture

<u>Graduate Work</u>



<u>Career</u>

NAGC At A Glance

"To translate scientific discoveries into solutions for production agriculture, functional foods, and bioenergy."

Staff

40+ years of Lab Experience

ISO Accredited Testing Lab

NAGC

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

Collaborators

NORTH DAKOTA DEPARTMENT of AGRICULTURE

MDAR MASSACHUSETTS DEPARTMENT OF AGRICULTURAL RESOURCES

USDA Agricultural Research Service

UDAF

VERMONT AGENCY OF AGRICULTURE, FOOD & MARKETS

ApisProtect

NDSU NORTH DAKOTA STATE UNIVERSITY

What motivates us

- **Bridging the gap** between research and practical applications in biotechnology
- **Diagnostics is lagging** in agriculture compared to livestock & human health
- **Pest & pathogens** are top threats to crop supplies and products

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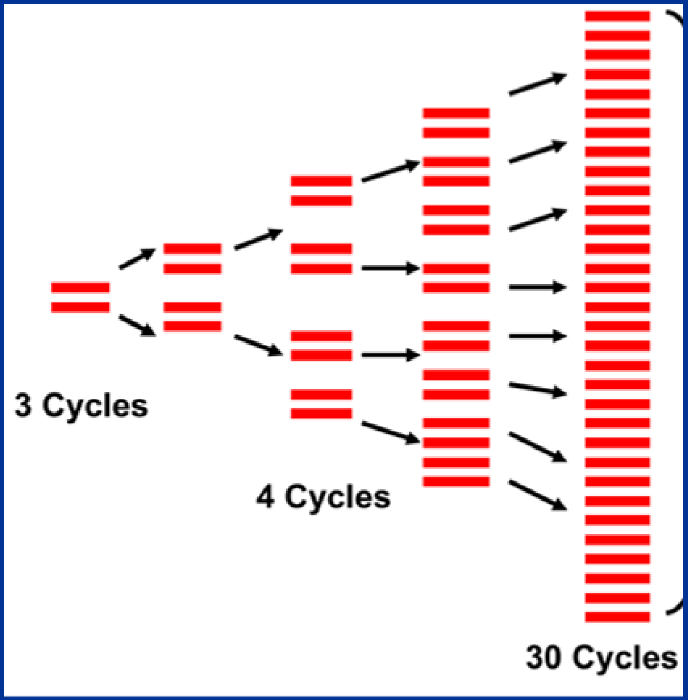
PCR-based Diagnostics

Polymerase Chain Reaction (PCR)

Molecular copier for small segments of DNA

Diagnostic tool to:

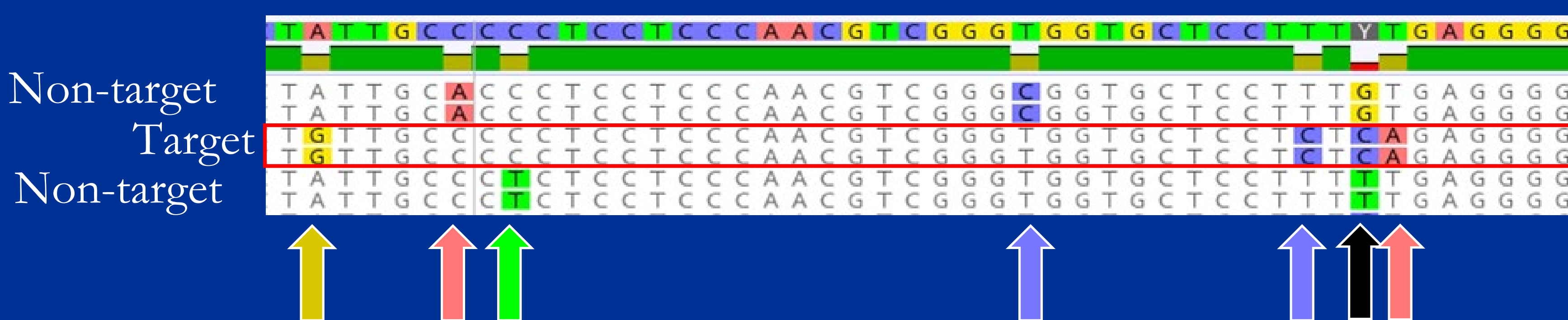
- Detect and quantify pathogens
- Detect unique traits in organisms



Overview of PCR-based Test Development

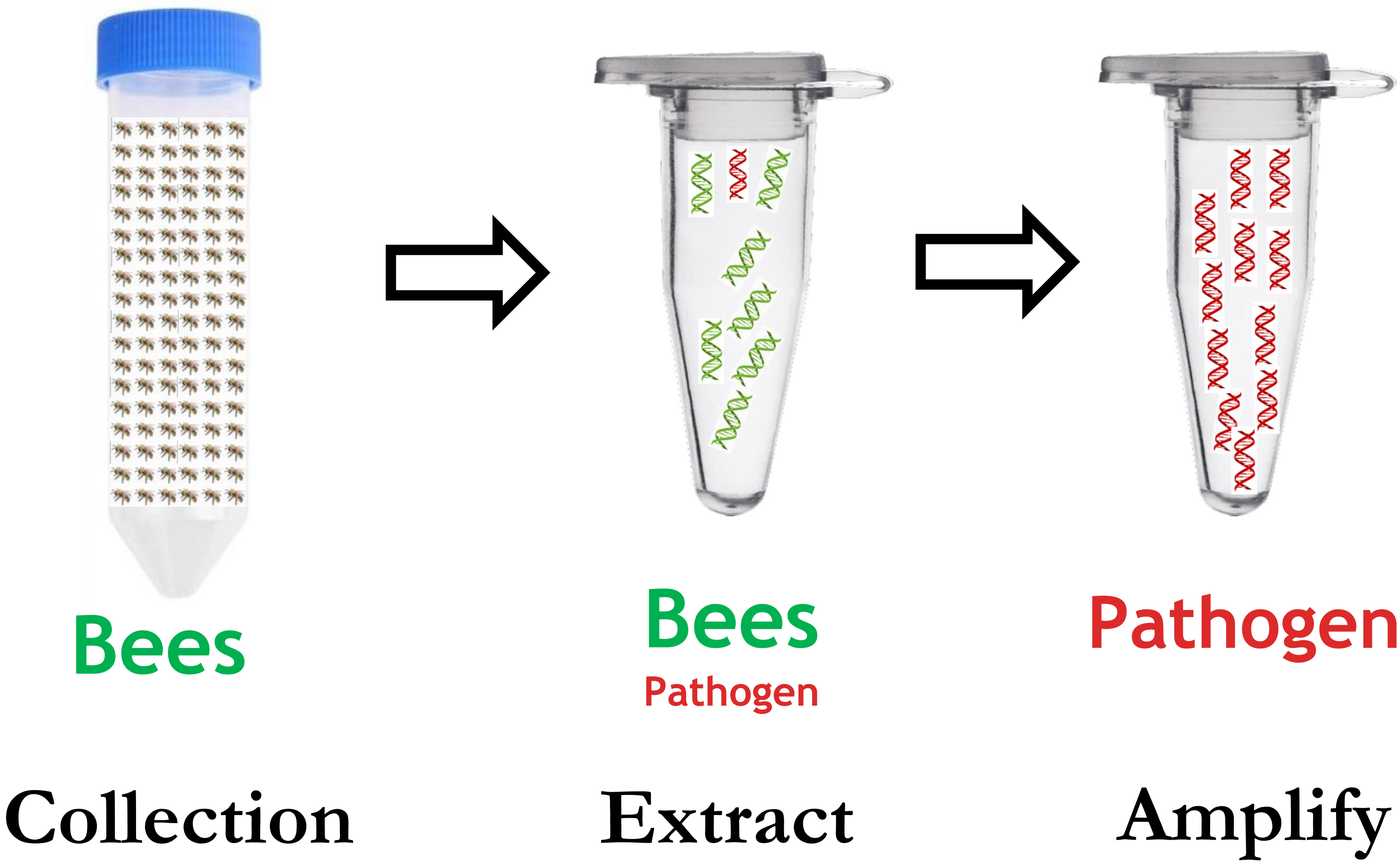
Identify genetic target	Find ***unique*** genomic regions	Design primers to amplify target with **PCR**	Use detection aids (probes) with light-sensitive equipment

DNA Sequence Alignment

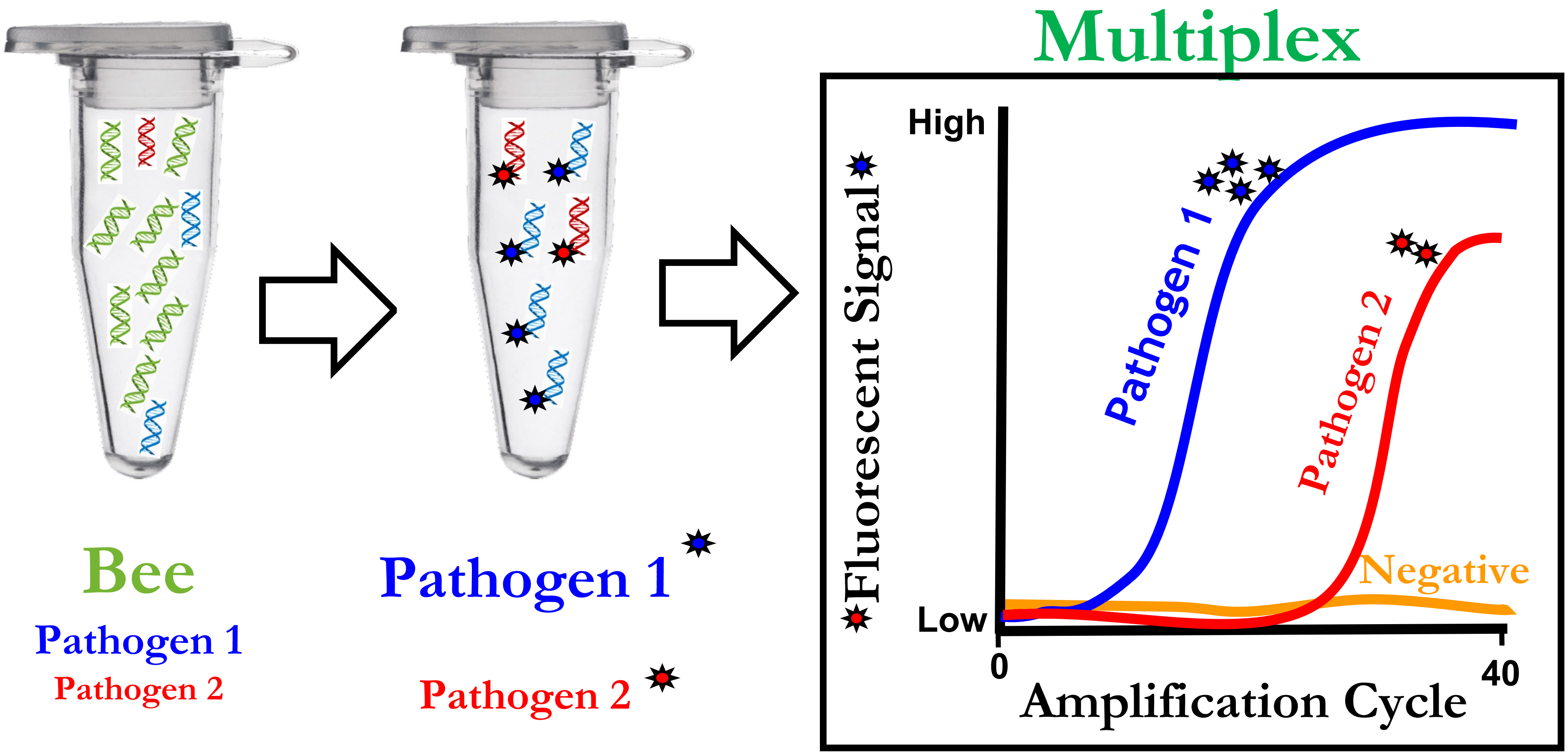


Arrows = DNA differences between target and non-targets

PCR-based Diagnostics

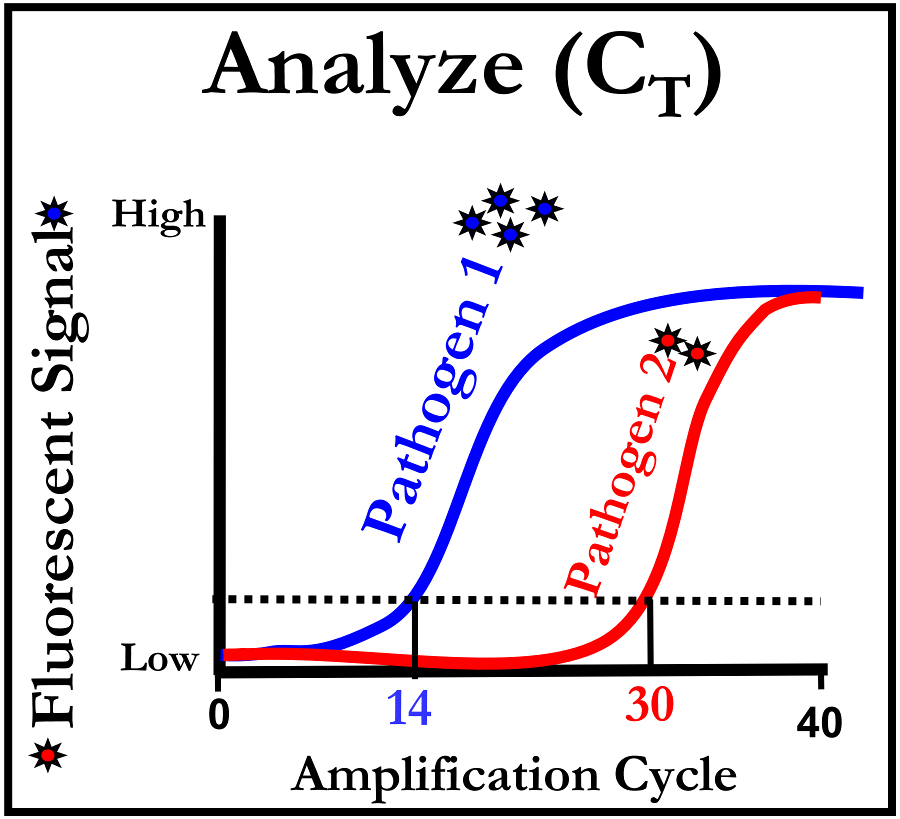


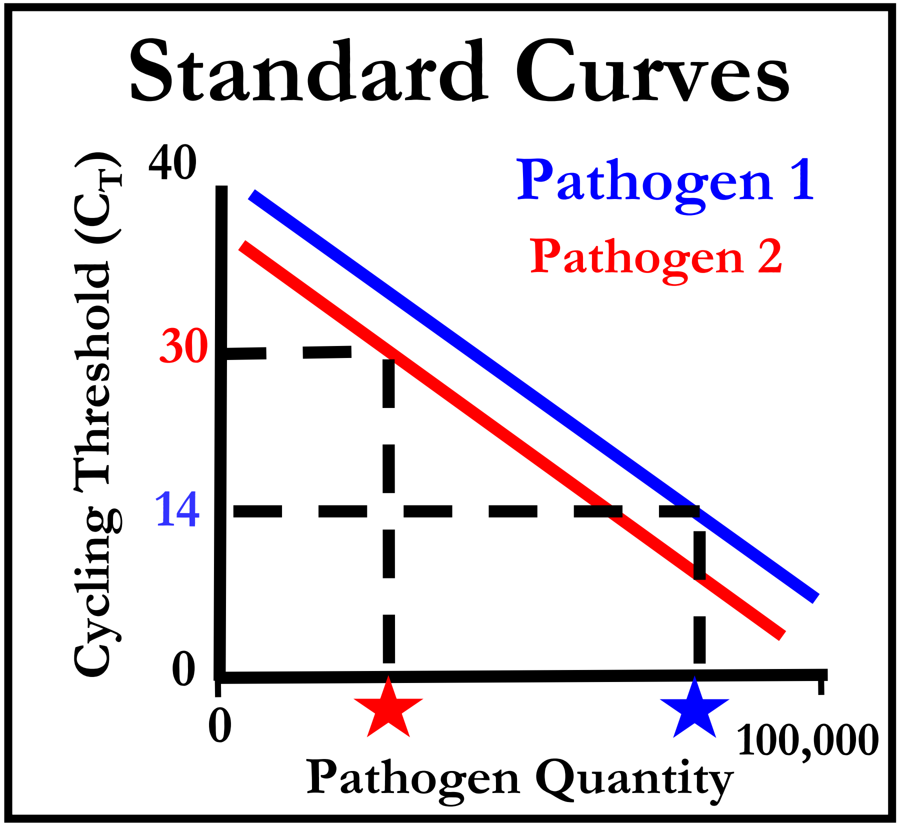
PCR-based Diagnostics



Extract Amplify Analyze

PCR-based Diagnostics





High-throughput

Diagnostic Research at NAGC

Bees

Crops

Weeds

Honey Bee Crisis

OPEN ACCESS Freely available online | PLoS BIOLOGY

Unsolved Mystery

What's Killing American Honey Bees?

Benjamin P. Oldroyd

NOTES AND COMMENTS

IBRA INTERNATIONAL BEE RESEARCH ASSOCIATION

Honey bee colony losses in Canada.

Robert W Currie[1*], Stephen F Pernal[2] and Ernesto Guzmán-Novoa[3]

ORIGINAL RESEARCH ARTICLE

INTERNATIONAL BEE RESEARCH ASSOCIATION

Declines of managed honey bees and beekeepers in Europe

Simon G Potts[1*], Stuart P M Roberts[1], Robin Dean[2], Gay Marris[3], Mike A Brown[3], Richard Jones[4], Peter Neumann[5,6,7] and Josef Settele[8]

Review

Cell PRESS

Bees brought to their knees: microbes affecting honey bee health

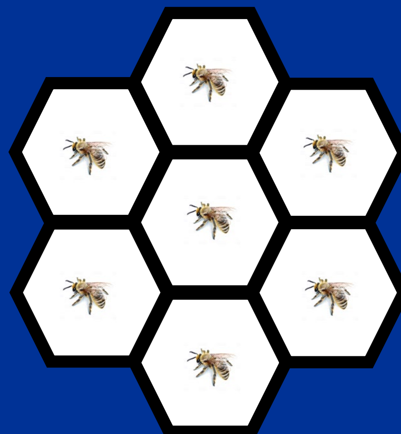
Jay D. Evans and Ryan S. Schwarz

United States Department of Agriculture (USDA)–Agricultural Research Service (ARS) Bee Research Laboratory, Beltsville Agricultural Research Center (BARC) East Building 476, Beltsville, MD 20705, USA

Multiple factors contribute to colony persistence

Nutrition

Genetics



Weather

Pathogens

Pesticides

Mites

Management

Honey Bee Pathogen Panel

Dicistroviridae

- Acute Bee Paralysis Virus (ABPV)
- Black Queen Cell Virus (BQCV)
- Israeli Acute Bee Paralysis Virus (IABPV)
- Kashmir Bee Virus (KBV)

Iflaviridae

- Deformed Wing Virus-A (DWV-A)
- Deformed Wing Virus-B (DWV-B)
- Sacbrood Virus (SBV)
- Slow Bee Paralysis Virus (SBPV)

Unclassified RNA viruses

- Chronic Bee Paralysis Virus (CBPV)
- Lake Sinai Virus-1 (LSV1)
- Lake Sinai Virus-2 (LSV2)

Bacteria

- *Melissococcus plutonius*
- *Paenibacillus larvae*

Fungi

- *Nosema ceranae*
- *Nosema apis*

Beekeepers move colonies between states

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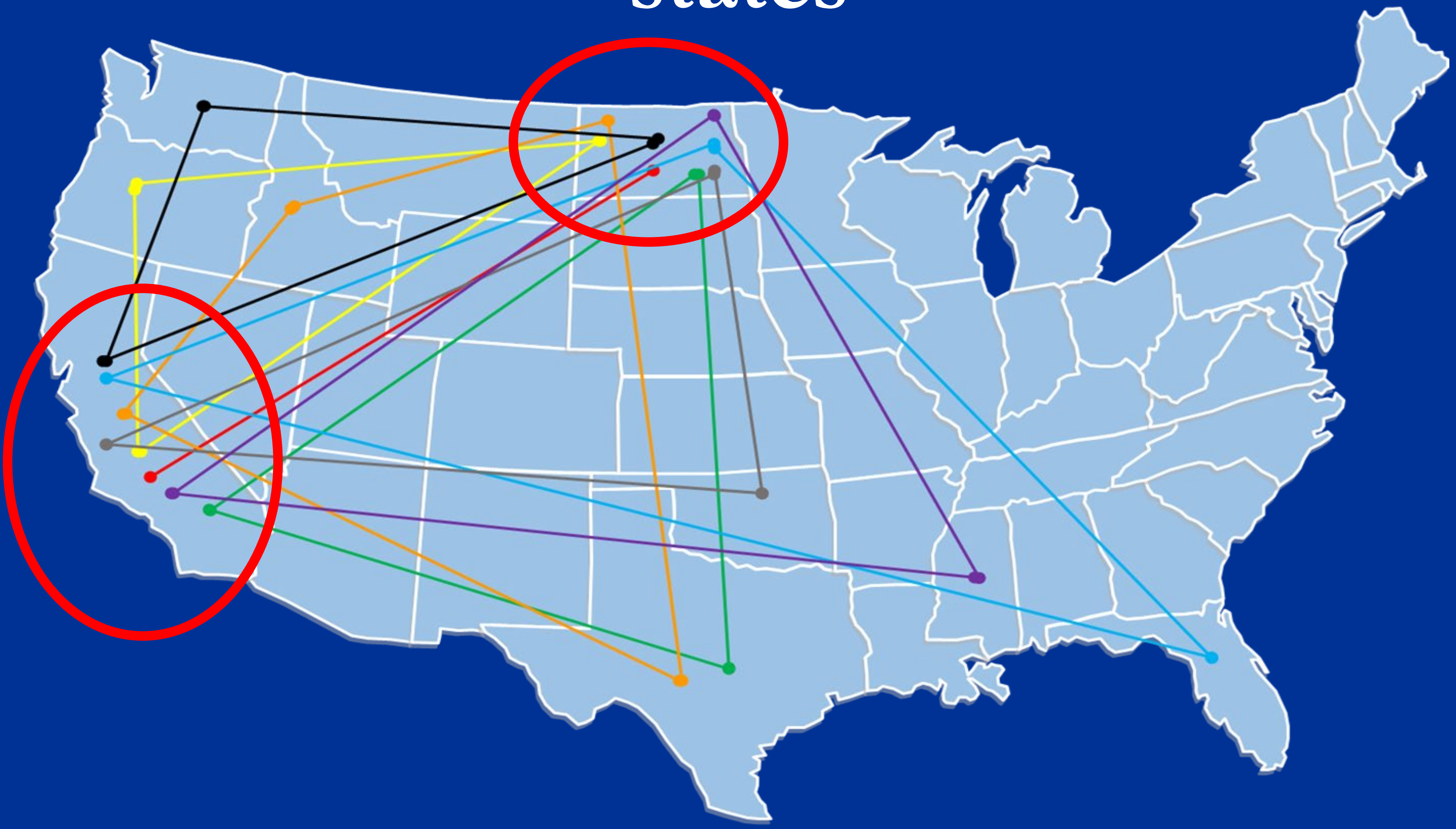

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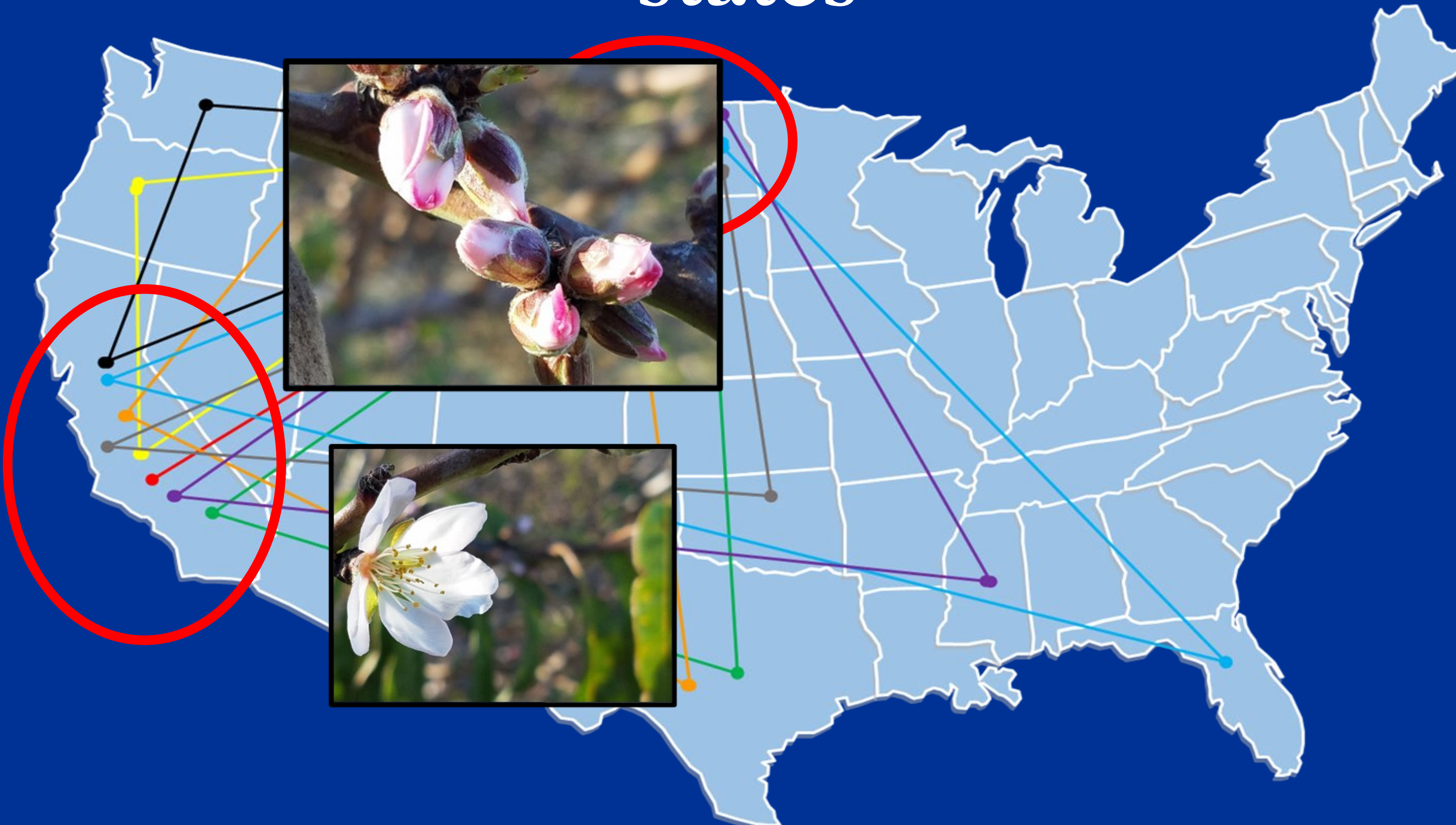
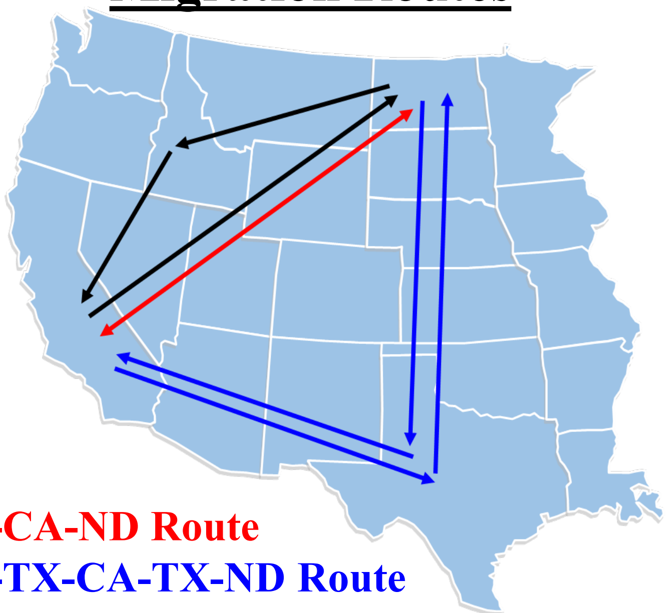


Honey Bee Research

Objectives. How is colony strength associated with pathogen loads in migrating honey bees?

Migration Routes



ND-CA-ND Route
ND-TX-CA-TX-ND Route
ND-Storage-CA-*-ND Route
*location of storage facility varied among operations

Study Overview – Colony Visits

Research Hive

- Feb 2019 (CA)
- June/July 2019 (ND)
- Aug/Sept 2019 (ND)
- Feb 2020 (CA)
- June/July 2020 (ND)
- Aug/Sept 2020 (ND)

Collected at Visit

Colony Strength
Varroa Mite Counts
Bees for Pathogen Panel

In partnership with

Objec n

patho

ND-CA-N

ND-TX-C

ND-Stora

*location of s

Visits

(CA)

2019 (ND)

2019 (ND)

(CA)

2020 (ND)

2020 (ND)

t Visit

rength

Counts

gen Panel

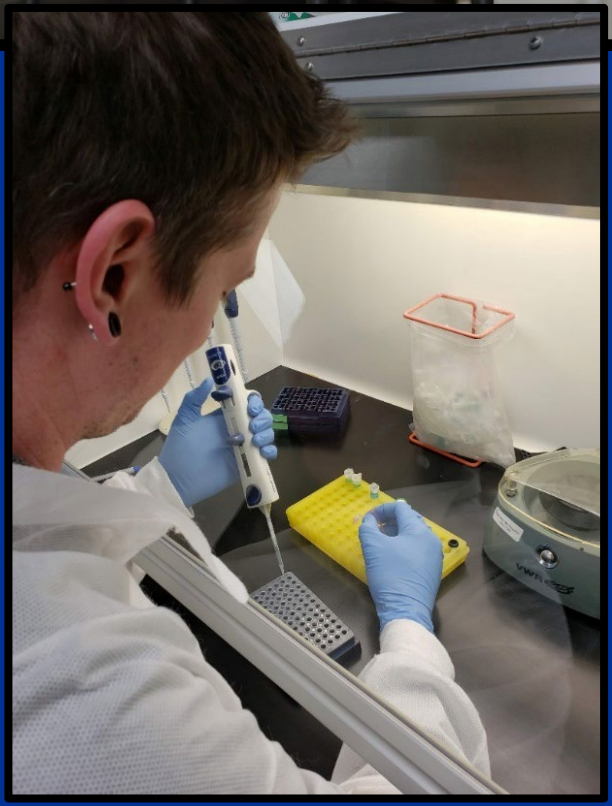
In partnership with

Field Work

CA

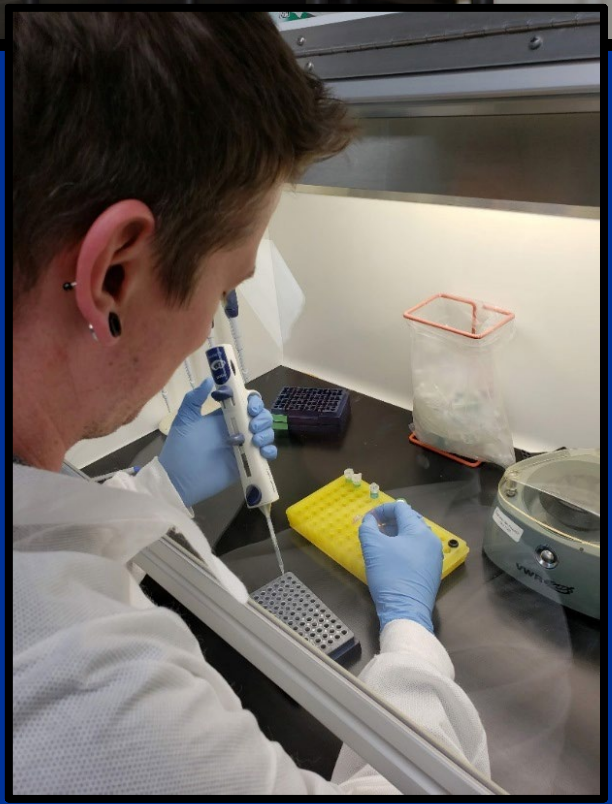
ND

Lab Work



Lab Work





Data Collected in 2019-20

Commercial Beekeeper Operations

- Eight operations (2,000 – 20,000 colonies)
- Sampling Events (Visits) = **957**

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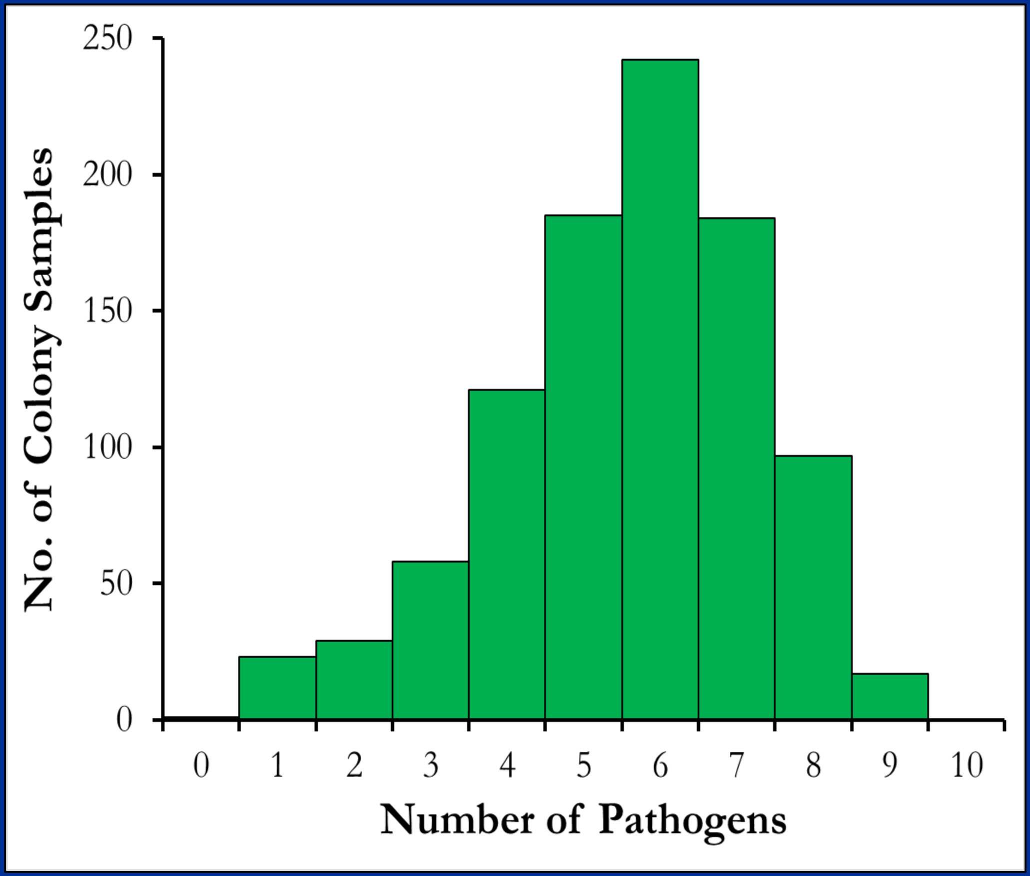
Variables

- Colony strength at visit (scale range 0-10)
- Colony loss (if known)
- Migration route (**Direct CA, TX, Storage**)
- Pathogen diagnosis and quantification at NAGC
  - Total Pathogen Tests **957 × 15 = 14,355**

Pathogen diversity across 957 colony visits



Average Pathogens/Colony Sample = 5.3

Top Seven Pathogens

Pathogen	**%**
Nosema ceranae	95
Sacbrood Virus	93
Black Queen Cell Virus	89
Deformed Wing Virus B	79
Deformed Wing Virus A	64
Israeli Acute Bee Paralysis Virus	25
Acute Bee Paralysis Virus	25

Factors Linked to Colony Strength

Factor	Result
IABPV	Greater loads in Weaker colonies
ABPV	Greater loads in Weaker colonies
Route	Storage route had Weaker colonies
Year	Stronger colonies in 2020 than 2019

Factor	Result
Pathogen Diversity	Greater diversity in Weaker colonies

Conclusions

Most colonies have sub-clinical infections

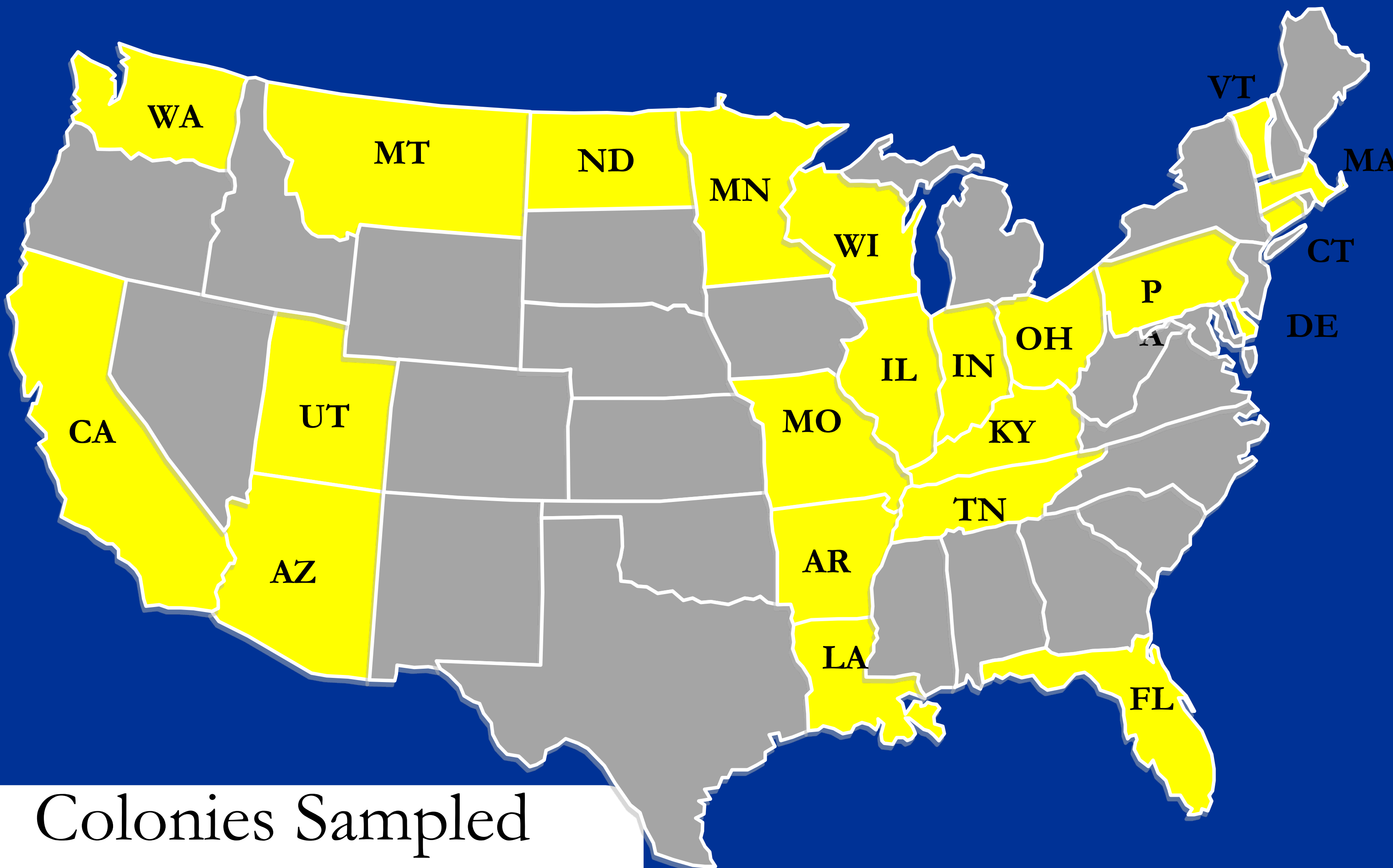
Weak colonies more likely to be infected with two viral pathogens: **ABPV & IABPV**

Weak colonies also had greater pathogen diversity

Transportation route affects colony strength

Monitoring pathogen levels can be a proxy for colony health and persistence.

Submitted Bee Samples (2016-20)



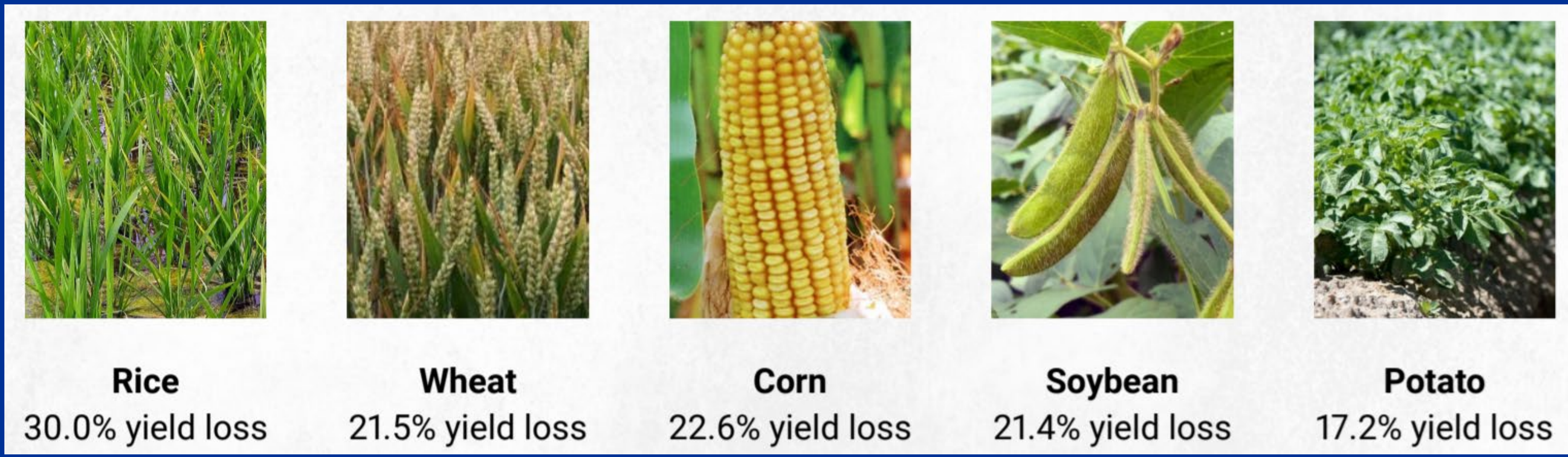
Colonies Sampled

2000+

States **22**

Pest and Pathogen Diagnostics in Crops

Pathogens & Pests contribute to dramatic worldwide yield losses

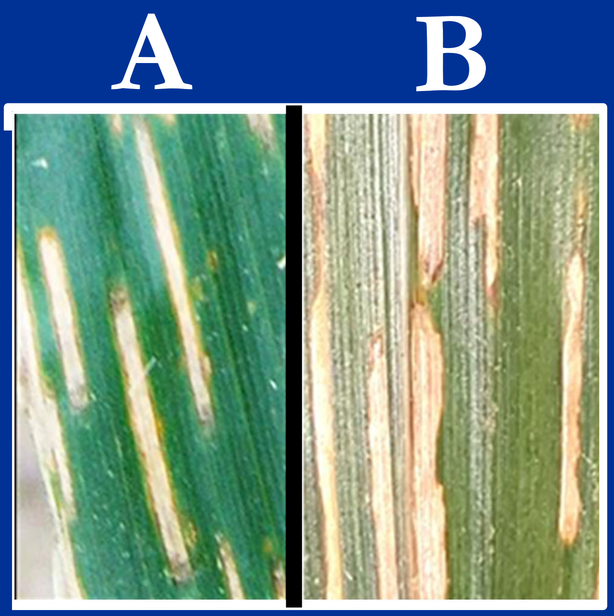


These 5 crops account for **half** of global human calorie intake!

Source: Savary et al. 2019. Nature Ecol & Evol, 3, 430-439

Current issues with diagnostics of crop diseases

1) **Symptom-based diagnosis in the field is difficult**



"Symptoms may look similar to other common diseases, <u>sometimes causing confusion and misdiagnoses</u>." – CropWatch, UNL

"Symptoms vary by hybrid susceptibility. Hybrids <u>may not experience the characteristic lesion</u>." – Purdue University Ext.

Current issues with diagnostics of crop diseases

2) **Reliance on traditional lab techniques that are less sensitive**

- Fresh sample required
- Unable to test environment (soil or residue)

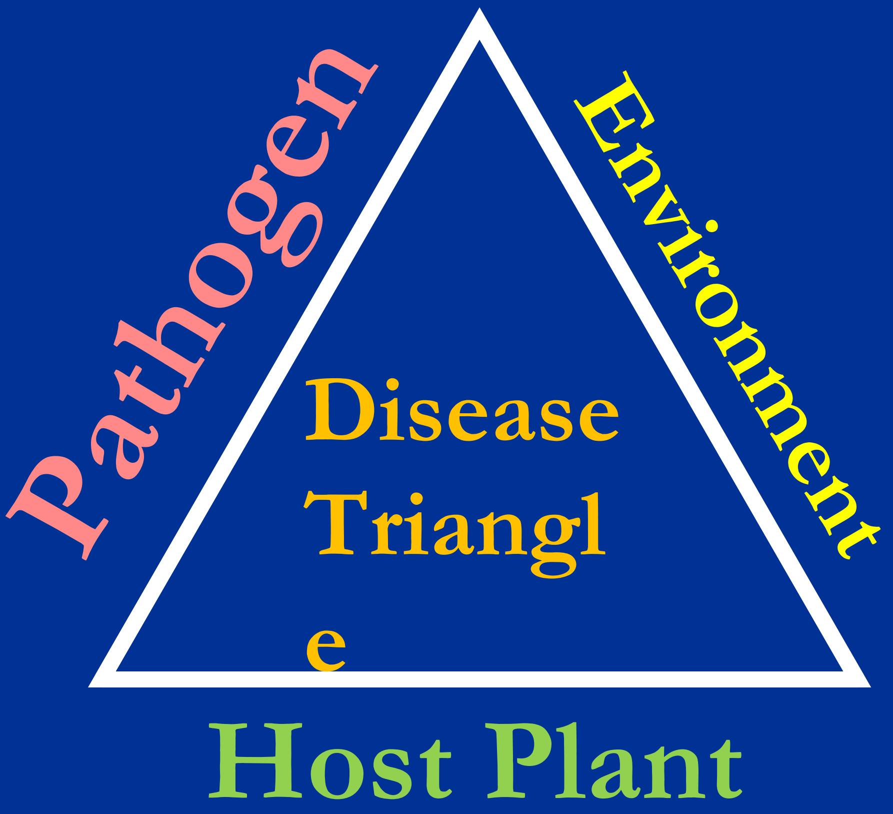
Surveillance for Disease risk relies on weather data

Weather data

- Easiest to collect
- Only 1 side of the disease triangle

Pathogen data

- Increases resolution of disease risk



National Predictive Modeling Tool Initiative

New in 2020

27 Collaborating University and Research Institutions

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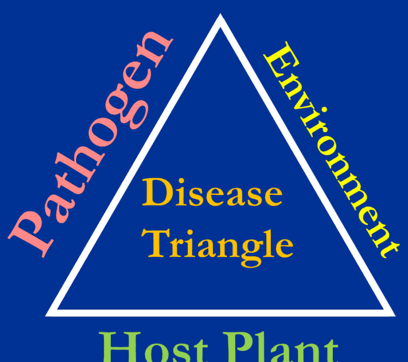
Inputs



Soil, residue, and spore samples

National Predictive Modeling Tool Initiative

New in 2020

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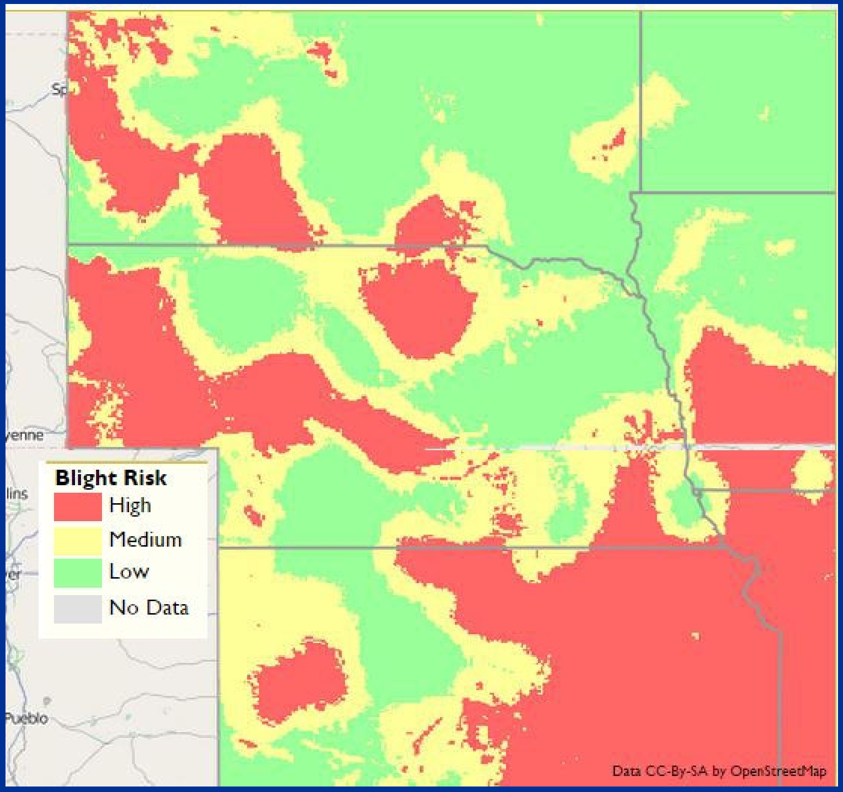
Output

Heat Map



Weed Diagnostics

Herbicide resistance is an increasing issue

<u>Unrestrained</u> weed growth would reduce crop yield by 50% across US and Canada

[The bill = $43 billion annually]

Photo – University of Missouri

Soltani Weed Tech 2017 31:148-154

Amaranths (Pigweeds)

Prolific seed producer – 100,000s of seeds

Dispersed by wildlife, flooding, humans

Fast growth rate (up to 4 inches per day)

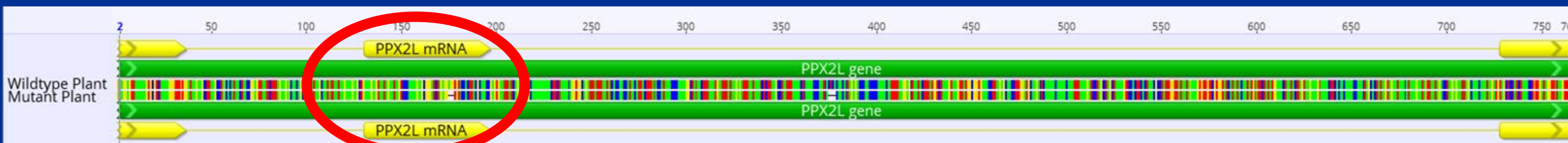
Up to 78% yield loss in soybeans

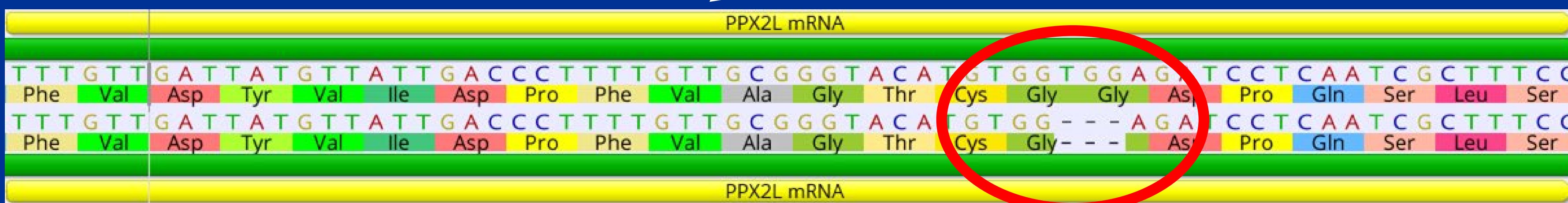
Herbicide Resistant

Pigweeds escaping herbicide treatments

Greenhouse Bioassays

Genetic basis for herbicide resistance *PPX2L* gene



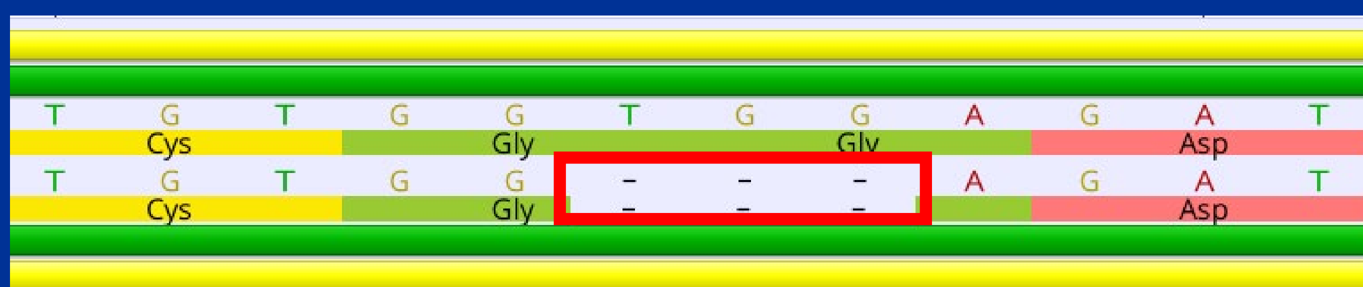


Missing Amino Acid in Mutant

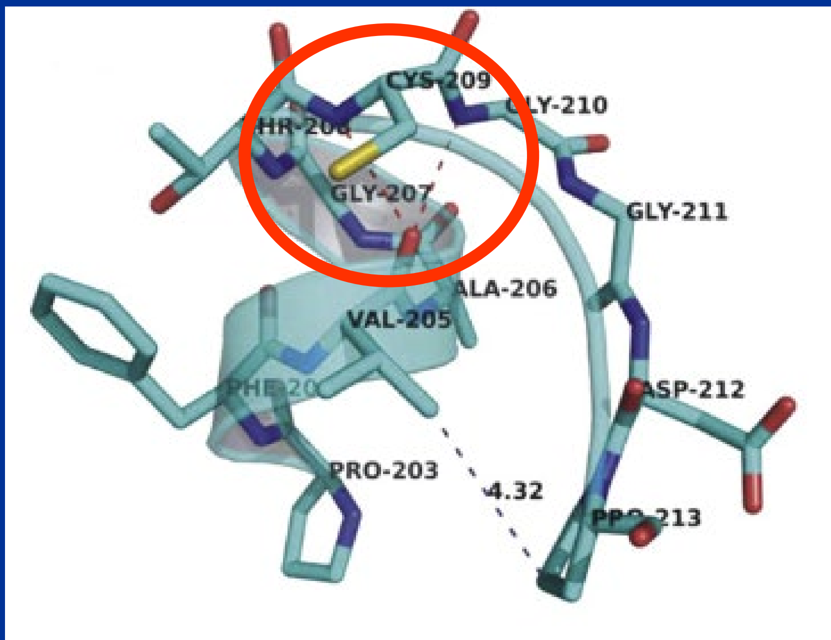
ΔG210

Wildtype Sequence

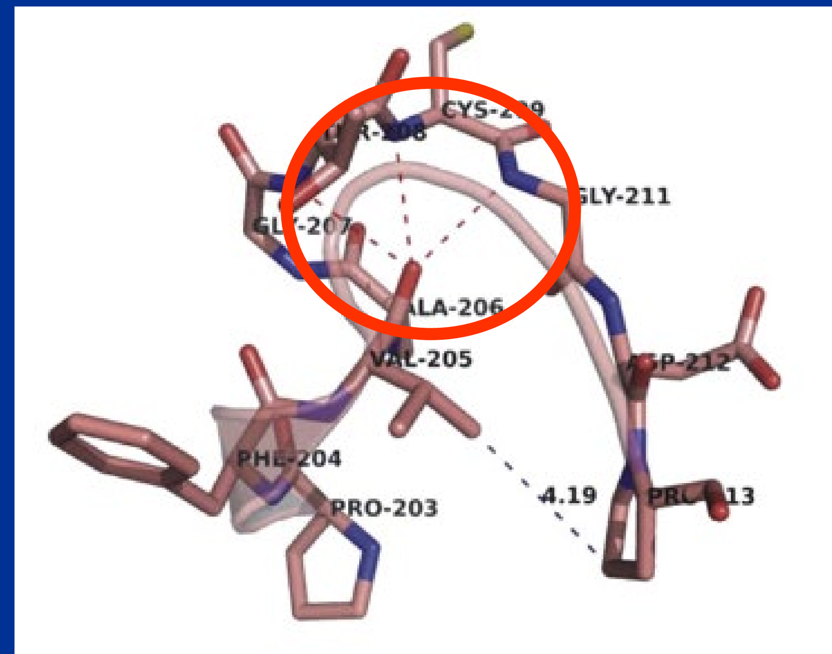
Mutant Sequence



Structure = Function



Wildtype - Only space for the herbicide molecule



Mutant – Large gap rendering the herbicide less effective

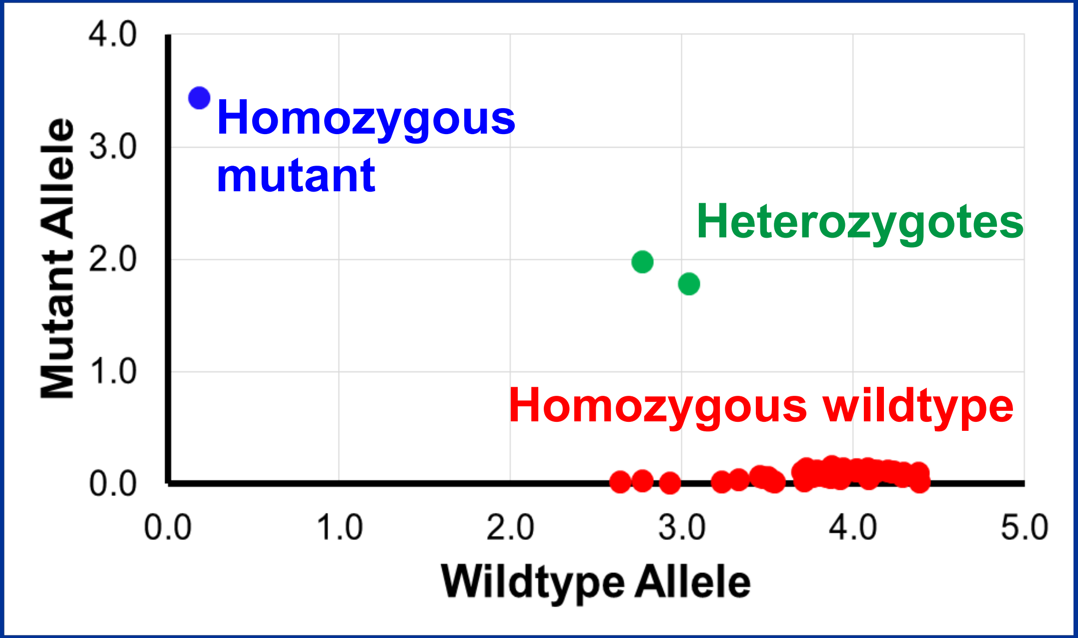
Dayon Handbook of Pesticide Toxicology 2010

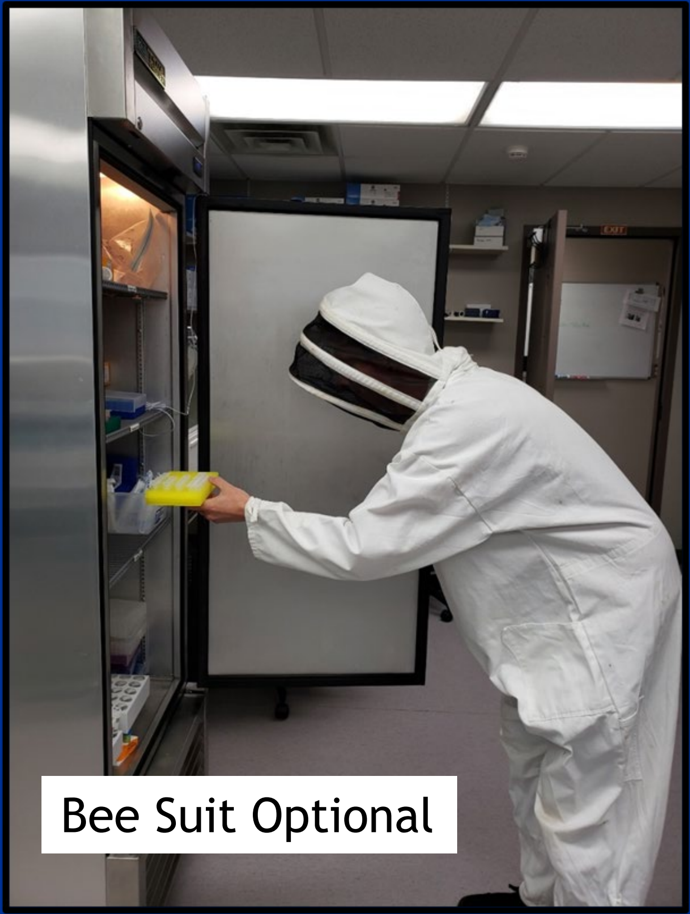
Genetic tests for herbicide resistance

Palmer amaranth & Waterhemp (Pigweeds)

PPO-Inhibitor Resistance (ΔG210)

Leaf samples





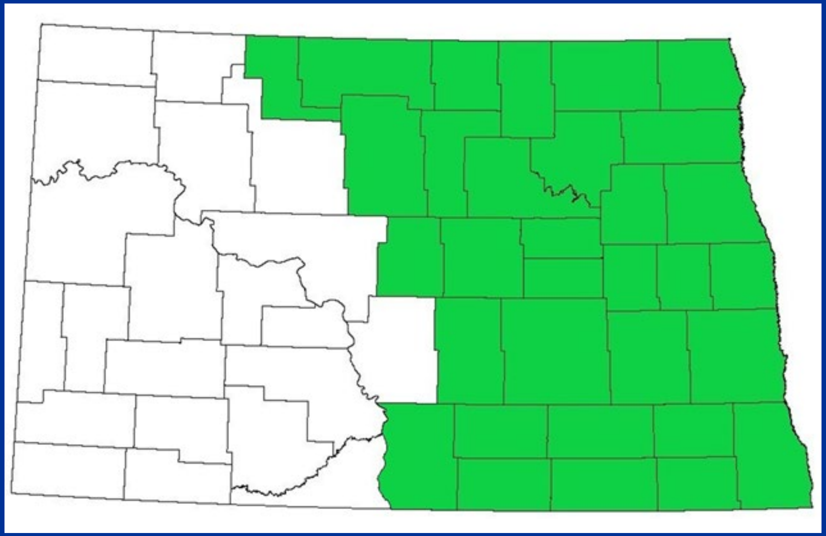
Bee Suit Optional

Upcoming Statewide Survey

Develop distribution maps for herbicide resistance in pigweeds

NAGC comprehensive panel

- PPO-inhibitors
- Glyphosate
- ALS-inhibitors



Waterhemp Distribution

Help producers avoid application of ineffective herbicides

Conclusions

- **Learning molecular protocols** create career opportunities than span disciplines.
  - Wildlife to Agriculture

- **PCR-based tests** can answer many pressing questions in disease and pest management

- **Strong need** for a workforce in agriculture diagnostics
  - Pests and pathogens are not going away!

Thank You!

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National Predictive Modeling Tool Initiative

www.agpmt.org