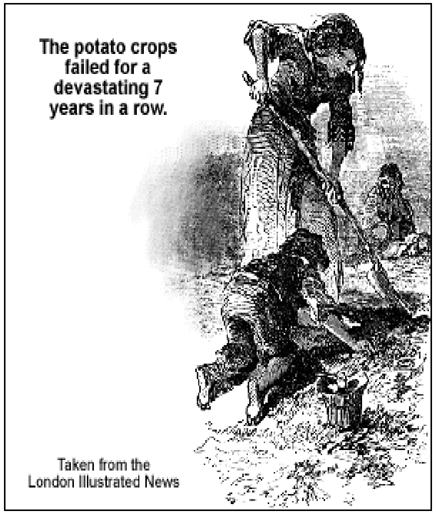
Predicting Future *Phytophthora* Outbreaks: New tools to identify emerging lineages and track spread.

Jean Beagle Ristaino

WNR Distinguished Professor of Plant Pathology

Emerging Plant Disease and Global Food Security Cluster



NC STATE UNIVERSITY Plant Sciences Initiative

Late blight re-emerging disease:

A constraint to potato production worldwide - impacts food security

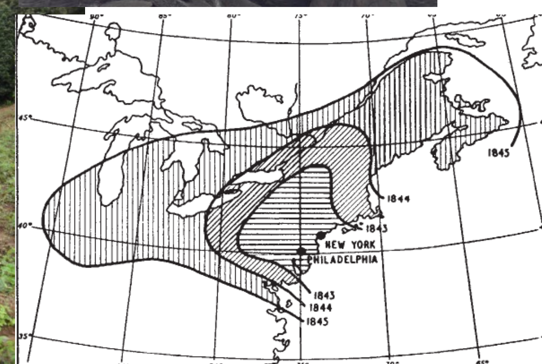


Fig. 1. Approximate extent of potato blight attacks in the United States and Canada during 1843–45 (after Stevens)

Has increased in incidence, geography, host range and virulence

PERSPECTIVE

The persistent threat of emerging plant disease pandemics to global food security

Jean B. Ristaino[a,1], Pamela K. Anderson[b,c], Daniel P. Bebber[d], Kate A. Brauman[e], Nik J. Cunniffe[f], Nina V. Fedoroff[g], Cambria Finegold[h], Karen A. Garrett[i,j], Christopher A. Gilligan[f], Christopher M. Jones[k], Michael D. Martin[l], Graham K. MacDonald[m], Patricia Neenan[n], Angela Records[o], David G. Schmale[p], Laura Tateosian[k], and Qingshan Wei[q]

Edited by Barbara Valent, Kansas State University, Manhattan, KS, and approved April 7, 2021 (received for review November 30, 2020)

Plant disease outbreaks are increasing and threaten food security for the vulnerable in many areas of the world. Now a global human pandemic is threatening the health of millions on our planet. A stable, nutritious food supply will be needed to lift people out of poverty and improve health outcomes. Plant diseases, both endemic and recently emerging, are spreading and exacerbated by climate change, transmission with global food trade networks, pathogen spillover, and evolution of new pathogen lineages. In order to tackle these grand challenges, a new set of tools that include disease surveillance and improved detection technologies including pathogen sensors and predictive modeling and data analytics are needed to prevent future outbreaks. Herein, we describe an integrated research agenda that could help mitigate future plant disease pandemics.

emerging plant disease | plant pathology | food security

June 8, 2021 | vol. 118 | no. 23 | e2022239118

PNAS

Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

PERSPECTIVE

- Need for surveillance
- Geospatial Analytics
- Earth Observations-remote sensing
- Sensors for early detection
- Pathogen Risk Modeling
- Data mining past and social media reports
- Phylogenomic surveillance
- Digital Delivery of information to stakeholders

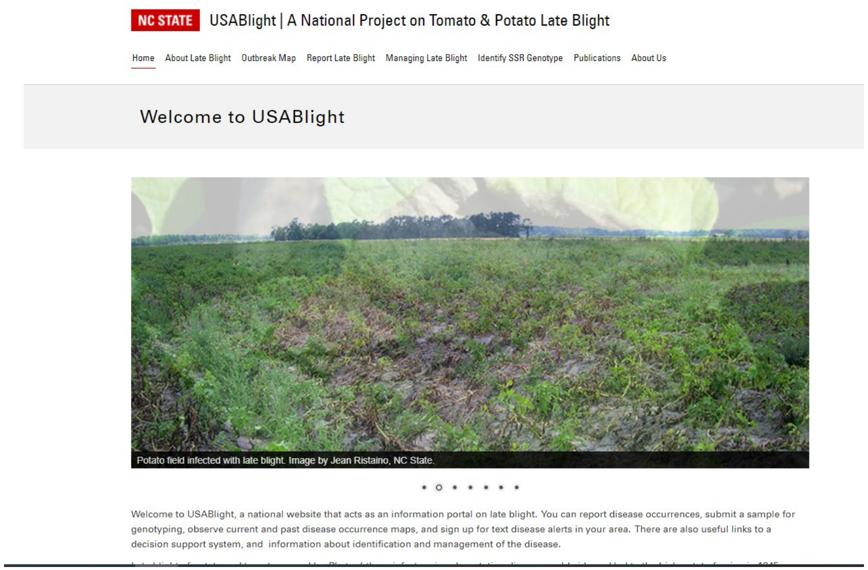
Overview of main points

- USABlight and has now transitioned into the Plant Aid Database (PaDB).
- Developed a global T BAS tool for Emerging *Phytophthora's* and SSR phylogeny and querying system to identify emerging *P. infestans* lineages
- Incorporating population genomics into forecasting systems to track spread of *P. infestans* globally and understand hotspots of 1c clade evolution
- Evolution of pathogen effectome in response to host R genes from 100 year of late blight evolution
- Developing a "Marple's" platform using targeted amplicon sequencing to monitor emerging traits of new lineages and changes within US-23 lineage
- Deploying and testing rapid field detection methods using LAMP assays for *Phytophthora infestans.*

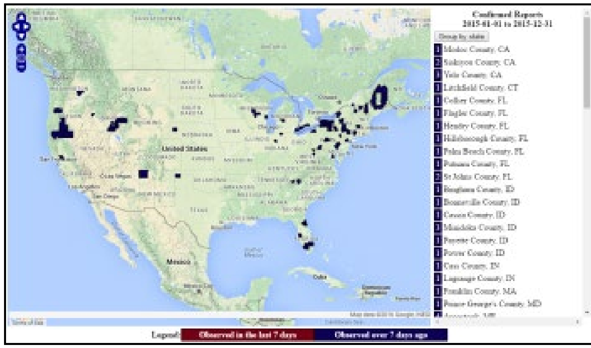
USABlight.org –Disease Surveillance

United States Department of Agriculture
National Institute of Food and Agriculture

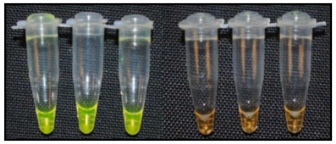
Sample Submission



Alerts and Mapping



New Diagnostics



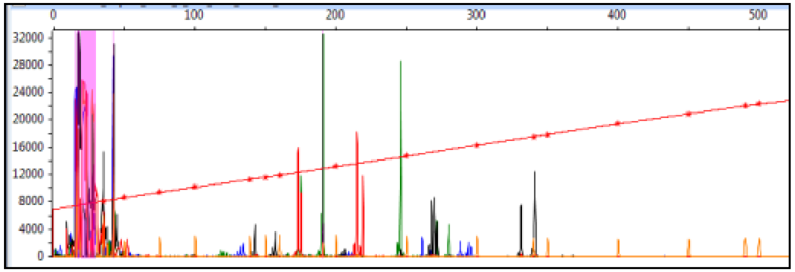
Fungicide Decision Support Tool

DSS Daily Email Report

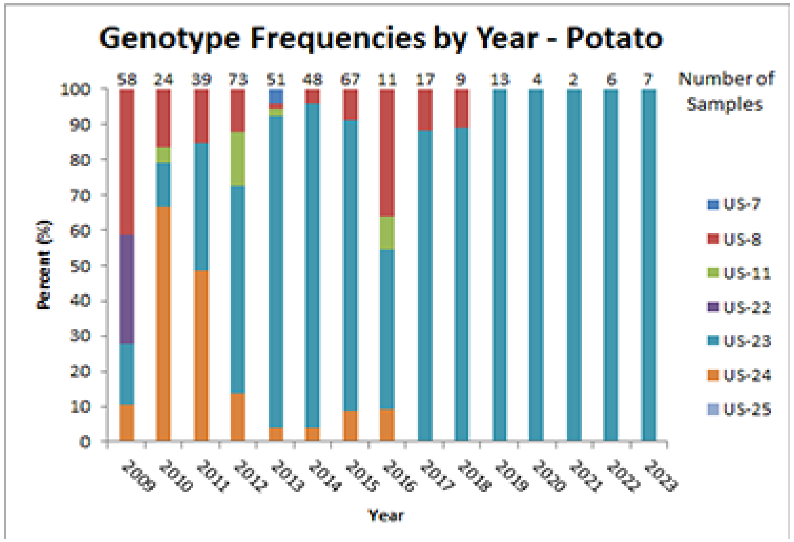
Mills River / ASHEVILLE

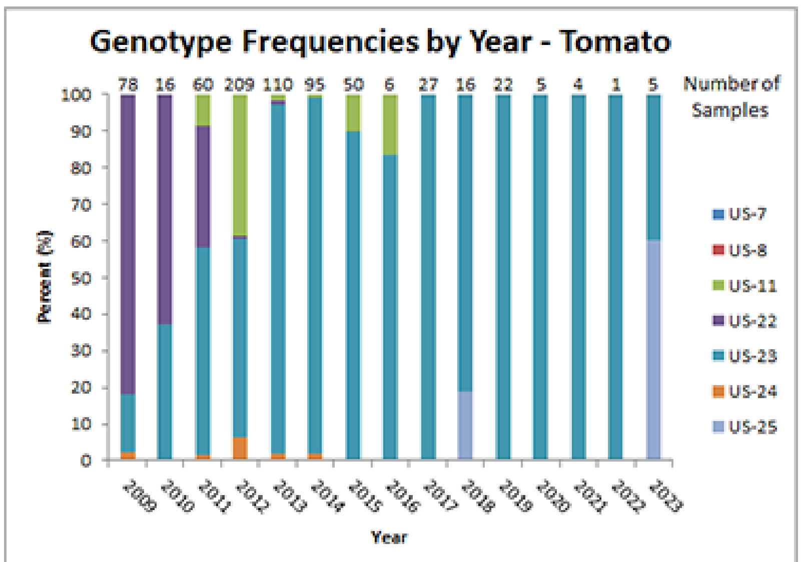
Date[1]	8/16	8/17	8/18	8/19	8/20	8/21	8/22	8/23
Blight Units	303	309	316	323	330	337	344	351
Fungicide Units	-91	-92	-94	-96	-97	-98	-99	-100
Key:								
below threshold								
blight unit threshold exceeded	>=30							
fungicide unit threshold exceeded	<=-15							

Genotyping

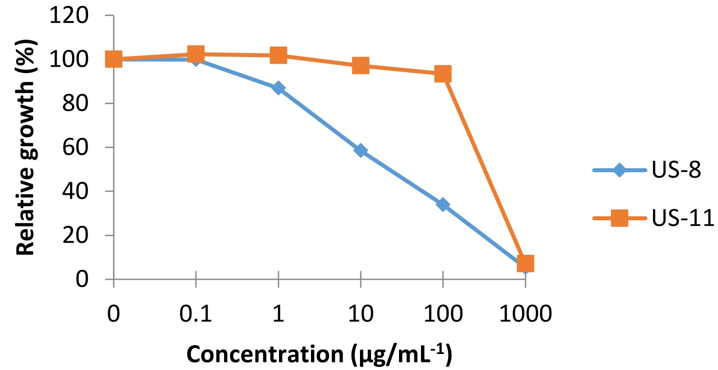


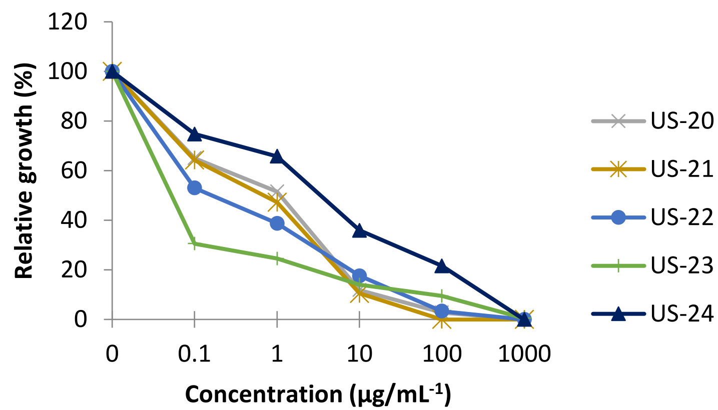
Evolution of lineages that differ in resistance to fungicides 2009-2023





- US-23 found on both tomato and potato -metalaxyl sensitive
- US-11 Tomato – west coast - mefenoxam resistant
- US-8 declined on potato - mefenoxam resistant



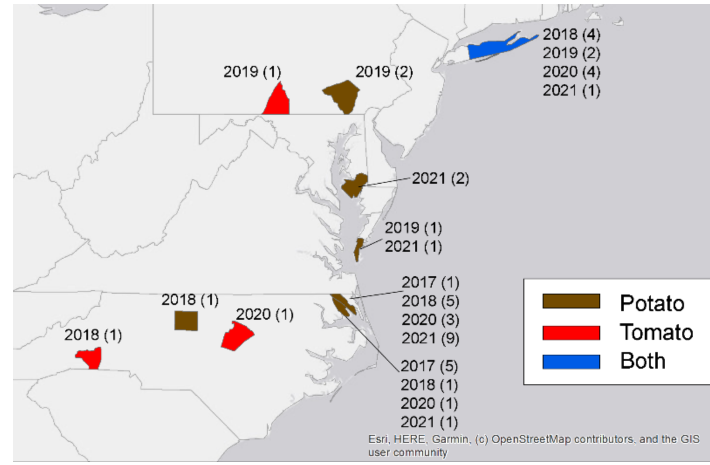


All lineages sensitive to azoxystrobin, cyazofamid, cymoxanil, fluopicolide, mandipropamid

-Saville, A. and Ristaino, J. B. 2019. Phytopathology 109:614-627.

-Saville, A. et al. 2015. Fungicide sensitivity of US genotypes of *Phytophthora infestans* (Mont.) de Bary to six oomycete-targeted compounds. Plant Dis. 99:659-666.

Late blight look-alike *Phytophthora nicotianae* – 27-30 C , less late blight on potatoes



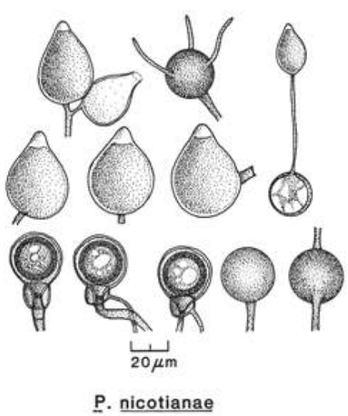
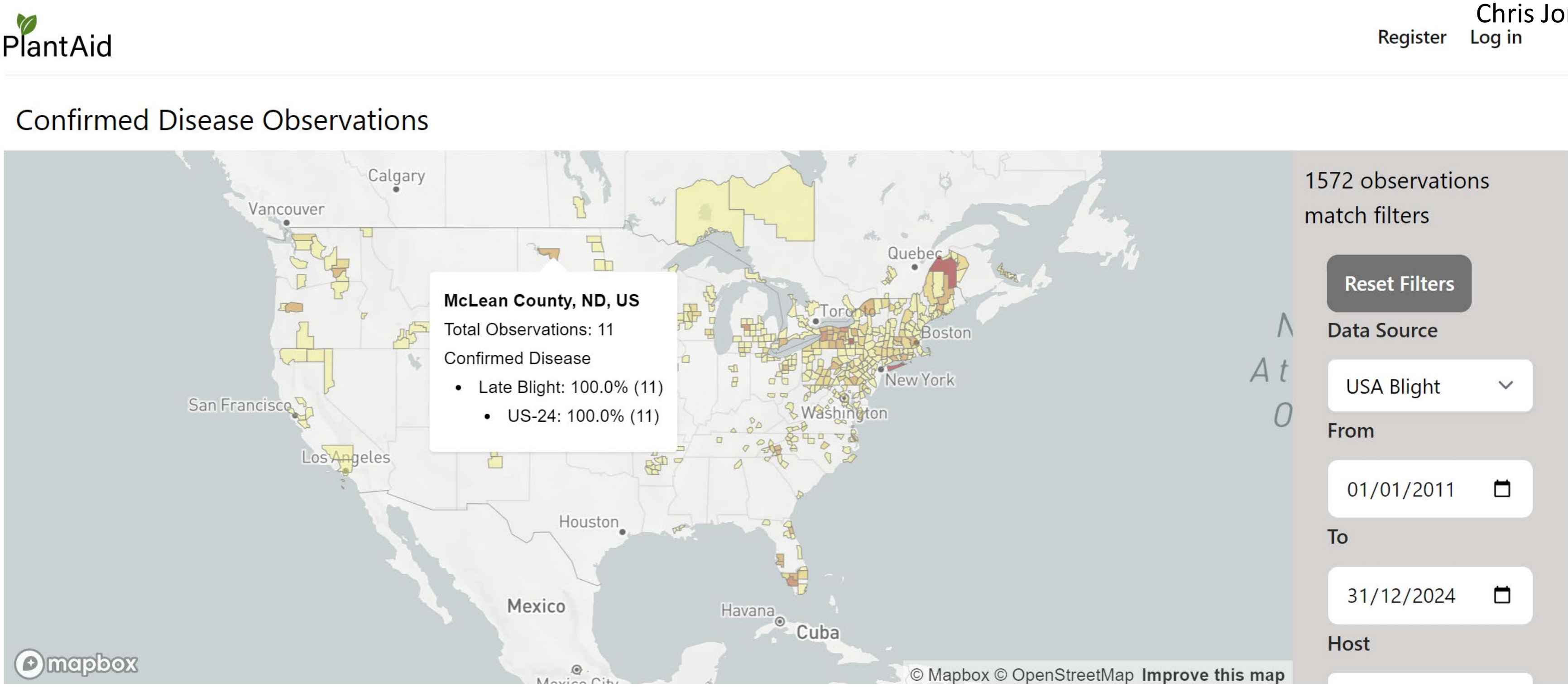


Figure 1. Morphology of *Phytophthora nicotianae* (=*Phytophthora parasitica*). Upper row, Papillate, ovoid sporangia; germinating chlamydospore. Middle row, Papillate, ovoid sporangia; germinating oospore. Lower row, Oogonia with amphigynous antheridia containing oospores; terminal and intercalary chlamydospores. (Courtesy A. Vaziri; Reproduced from Erwin and Ribeiro, 1996) Click image to see larger view.

PlantAid database

A new dashboard for late blight and other emerging plant diseases (Cucurbit downy mildew- *P. cubensis*, SOD - *P. ramorum*)

Chris Jones



- Center for Geospatial Analytics- now managing USABlight
- Incorporate more real time updates from in field detection sensors
- Incorporate population genomics tools into mapping platform

Developed a open T BASphylogenetic framework for “**Emerging *Phytopthoras*’** using multilocus genotyping

Genus level tool - uses multilocus sequences
196 species of *Phytophthora*

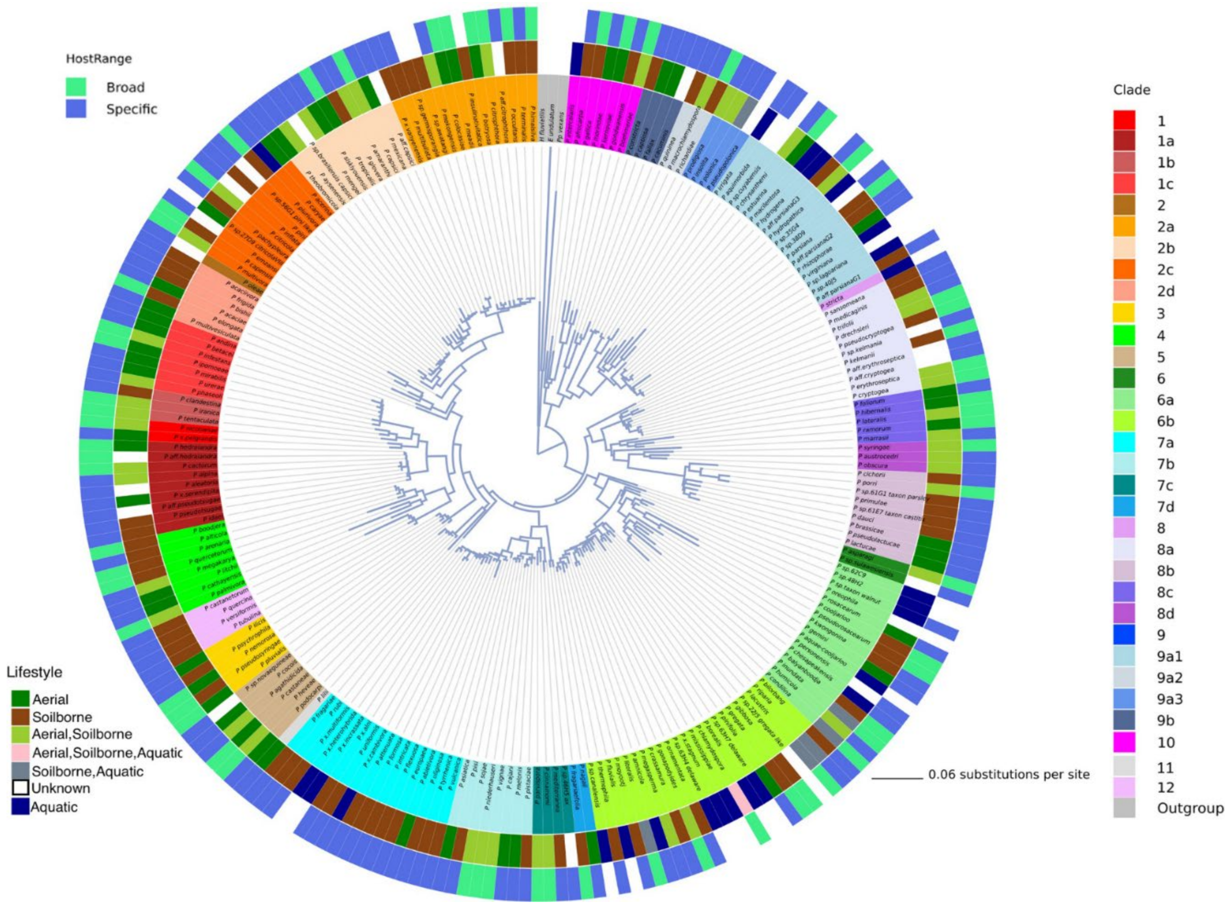


Allison Coomber   Ignazio Carbone Jean Ristaino

- 8 nuclear loci
- 192 species
- 33 informal taxa
- Inferred with RaxML

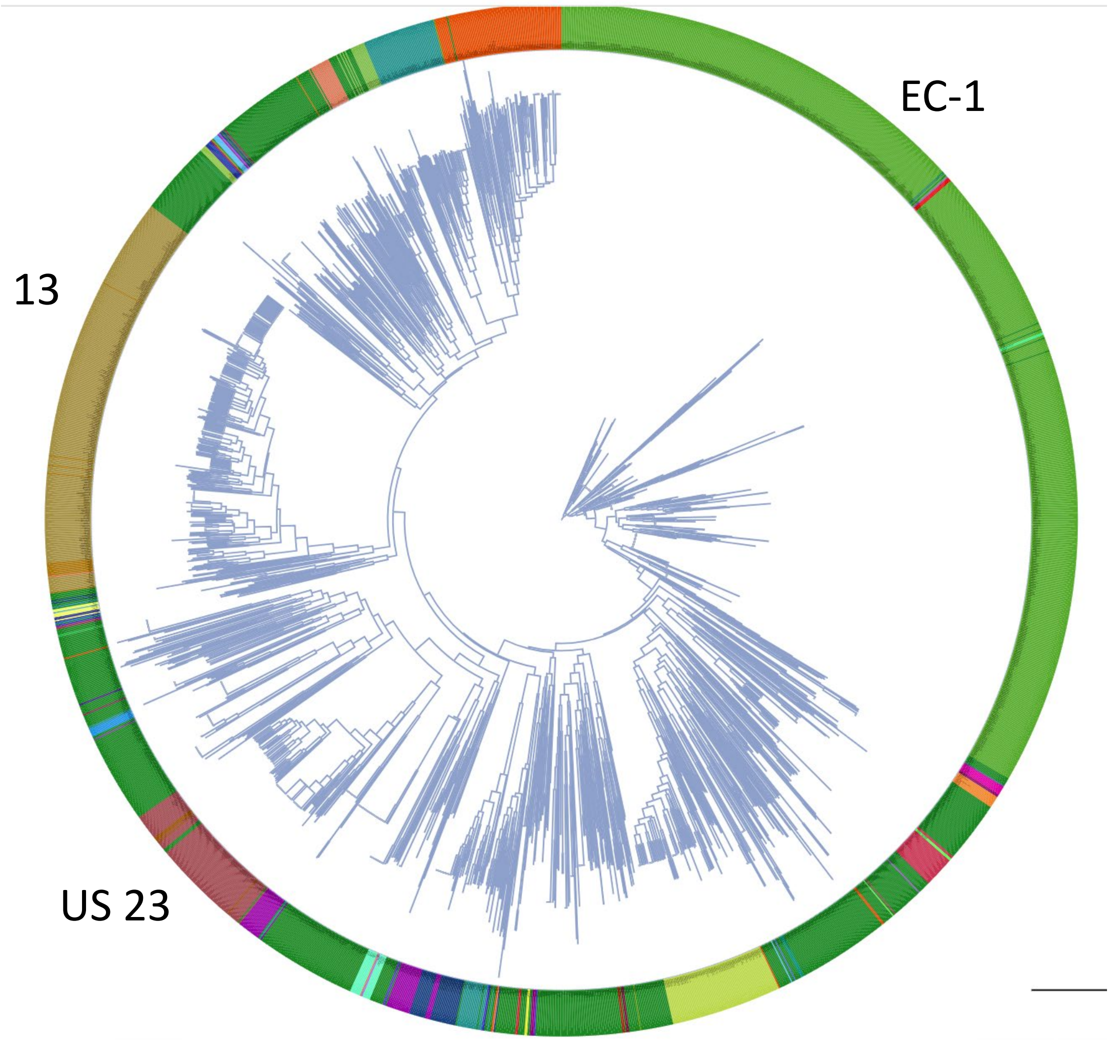
Loci included

28S
60SL10
Btub
EF1a
ENL
HS90
ITS
TigA



Allison Coomber, Amanda C. Saville, Ignazio Carbone, Jean B. Ristaino. 2023. An open access T-BAS phylogeny for emerging *Phytophthora* species. Plos One: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0283540

Developed a global T Bas SSR phylogeny and querying system to identify emerging *P. infestans* SSR lineages



https://tbas.cifr.ncsu.edu/tbas2_3/pages/tbas.php

David Cooke

Ignazio Carbone

Allison Coomber Jean Ristaino

Amanda Saville

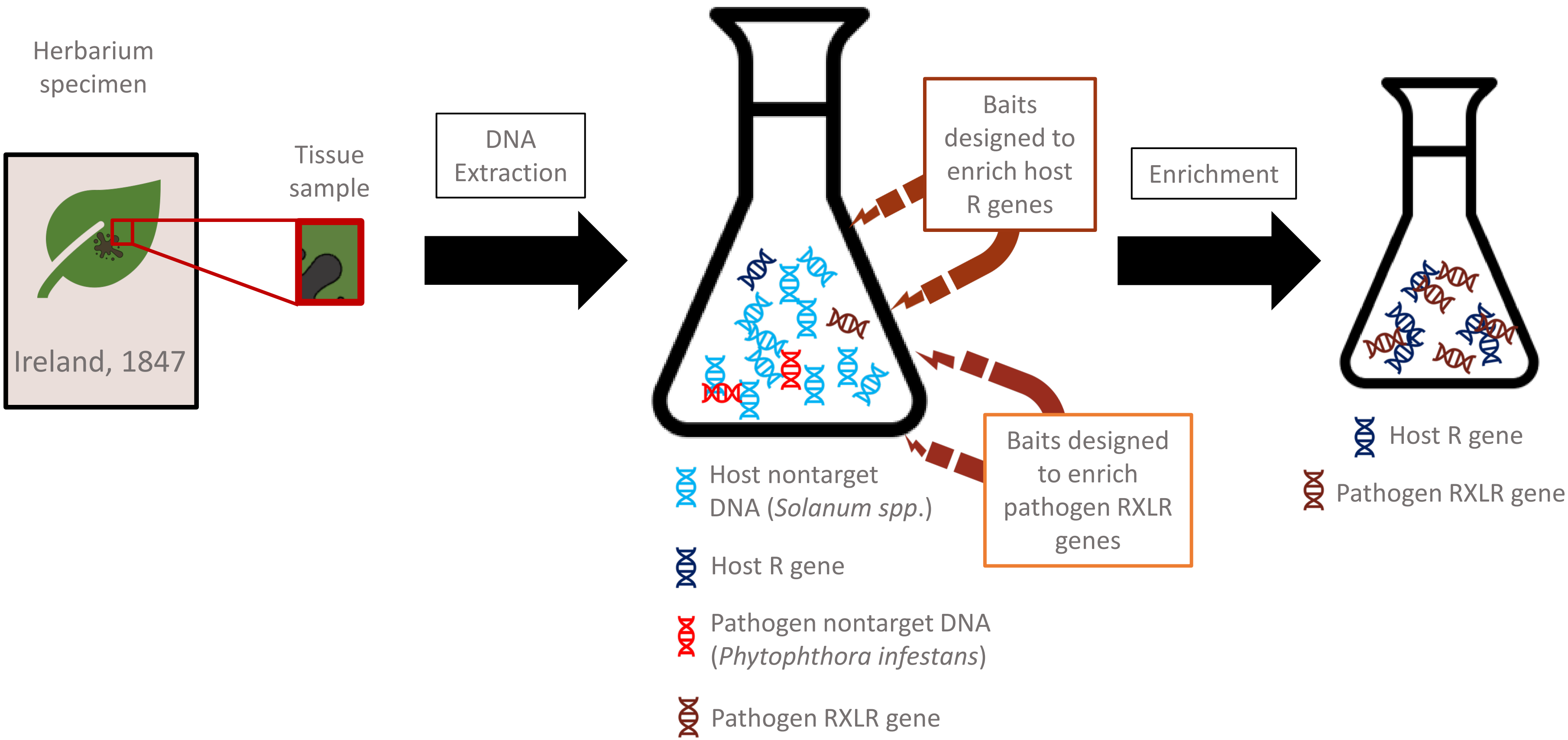
Open global *P. infestans* T BAS SSR phylogeny

Strain_name	Pi4B	Pi04	Long	PinfSSR6	Pi02	Lat	Country	Microsats	PinfSSR11	Pi63	PinfSSR2	PinfSSR4
PPA046	213/217/0	166/170/0	-75.75188889	244/258/0	266/266/0	-10.76494444	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPA044	213/217/0	166/170/0	-75.73250556	244/258/0	266/266/0	-10.76272222	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPA043	213/217/0	166/170/0	-75.73250556	244/258/0	266/266/0	-10.76272222	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPA041	213/217/0	166/170/0	-75.73250556	244/258/0	266/266/0	-10.76272222	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPA042	213/217/0	166/170/0	-75.73250556	244/258/0	266/266/0	-10.76272222	Peru		341/355/0	270/273/279	173/177/0	288/290/
PCA027	213/217/221	166/170/0	-78.415	244/250/0	266/266/0	-7.3125	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPI113	213/217/0	166/170/0	-79.48194444	244/254/0	266/266/0	-5.3055	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPI108	213/217/0	166/170/0	-79.56344444	244/254/0	266/266/0	-5.377305556	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPI107	213/217/0	166/170/0	-79.56344444	244/254/0	266/266/0	-5.377305556	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPI106	213/217/0	166/170/0	-79.56344444	244/254/0	266/266/0	-5.377305556	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPI105	213/217/0	166/170/0	-79.56344444	244/254/0	266/266/0	-5.377305556	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPI103	213/217/0	166/170/0	-79.56327778	244/254/0	266/266/0	-5.377222222	Peru		341/355/0	270/273/279	173/177/0	288/290/
PPI104	213/217/0	166/170/0	-79.56327778	244/254/0	266/266/0	-5.377222222	Peru		341/355/0	270/273/279	173/177/0	288/290/

Targeted effector and R gene sequencing

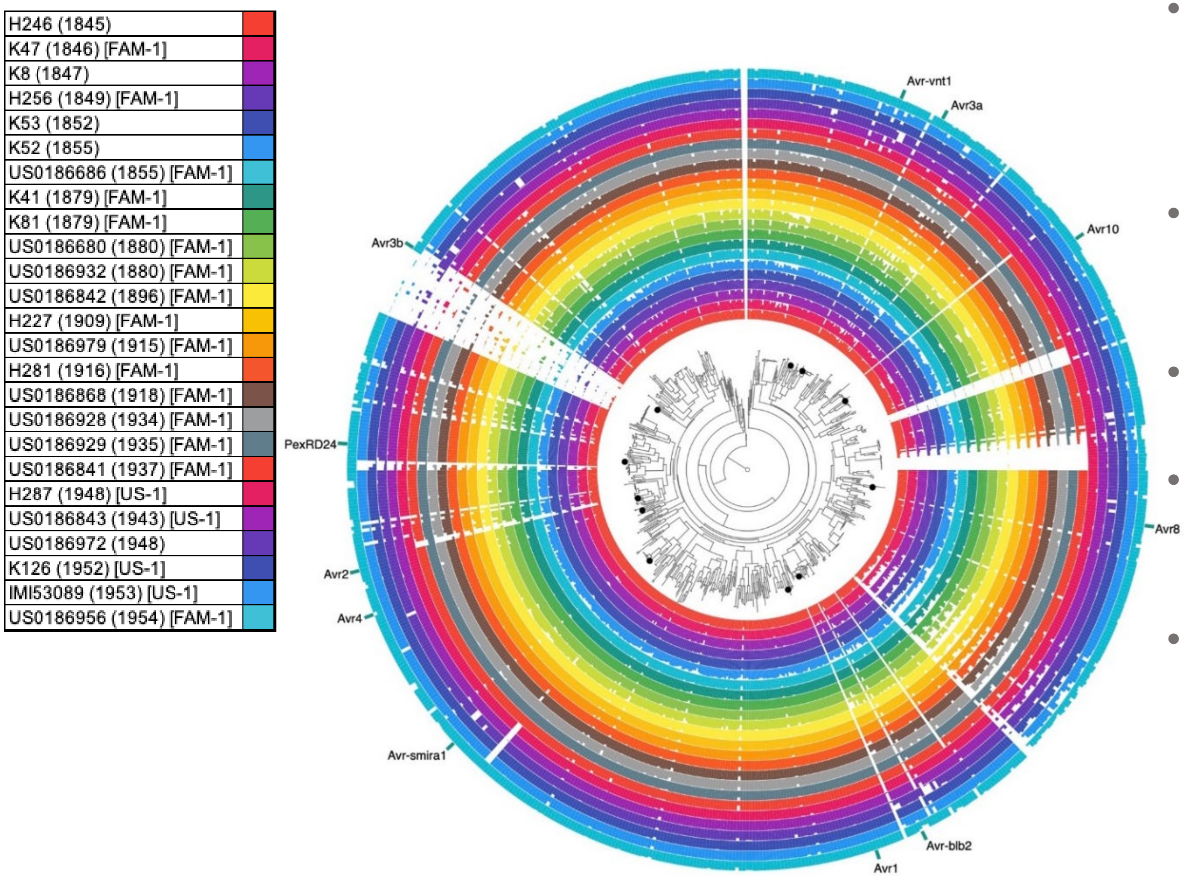


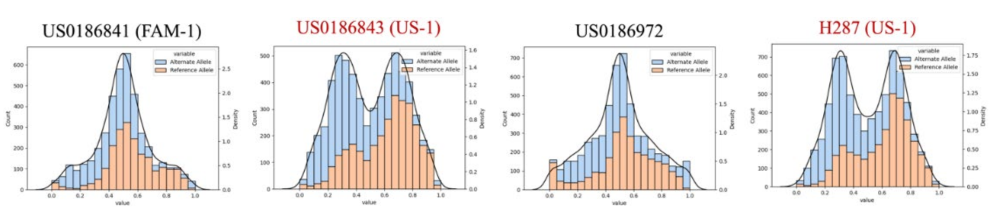
Allison Coomber, Amanda Saville, Jean Ristaino



Coomber, A. Saville, A., and Ristaino, J. 2024. Evolution of *Phytophthora infestans* on its Potato Host since the Irish Potato Famine . Nature Communications Pending

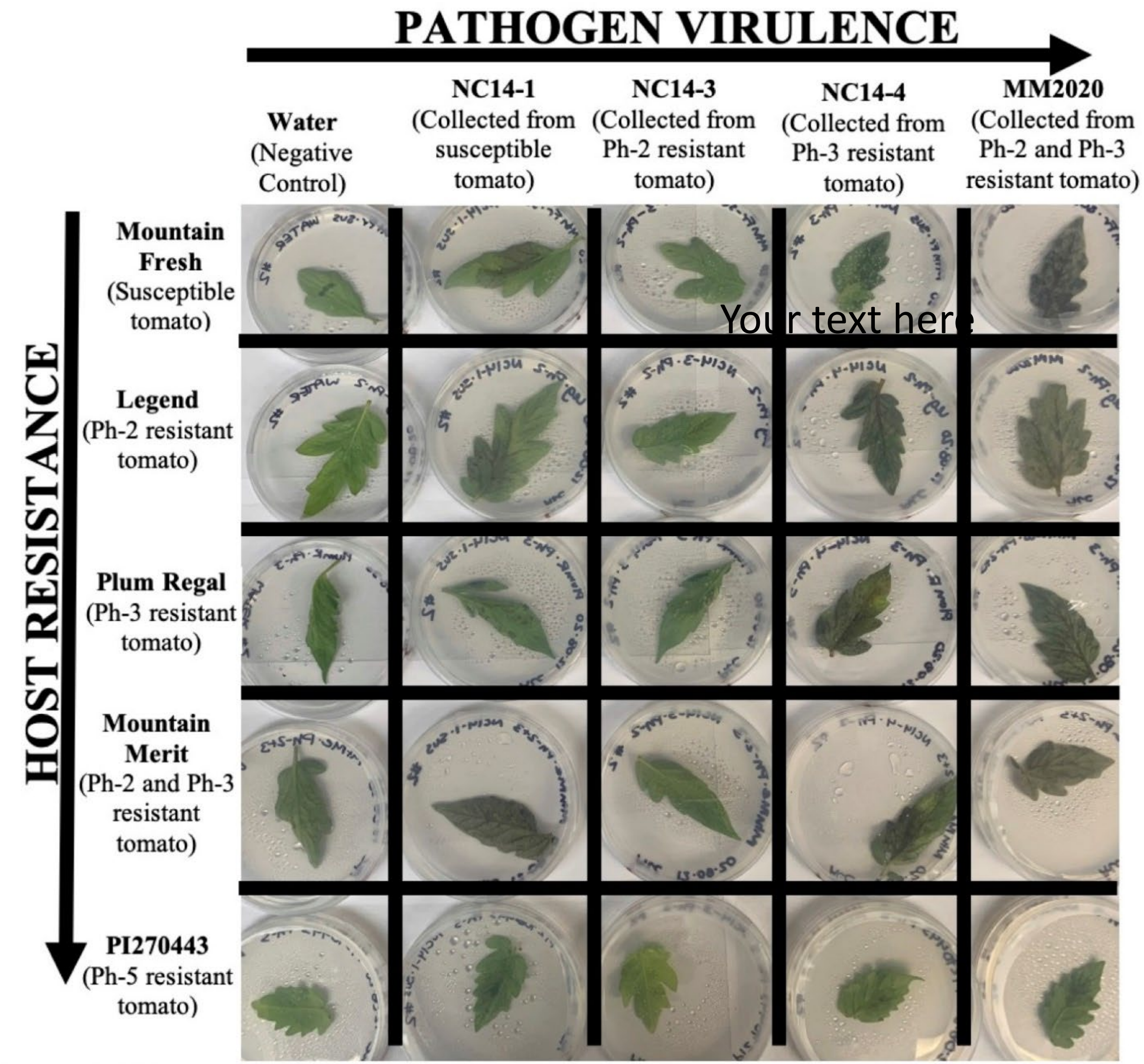
P. infestans effector evolution since the famine

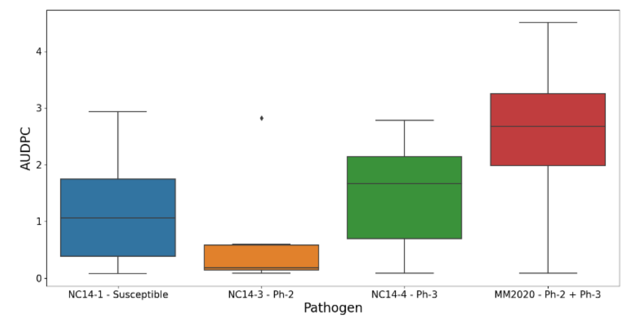




- **Expansion** of effectors over time
- Avr3b was the only cloned effector not present in Famine era samples but did appear in the mid 1900s
- Expansion coincides with the spread of genotype US-1 in 1930-50s
- FAM-1 lineages were diploid, US-1 triploid- ploidy changed
- AL virulent, **resistance-breaking allele of *Avr1* found in FAM 1 lineages**
- Although *R1* resistance had not yet been deployed in cultivated potatoes during the Famine era, **the FAM-1 lineage already possessed the ability to overcome the first resistance gene that would later be deployed by breeders.**

US-23 differs in virulence on tomato- Resistance breaking strain MM2020

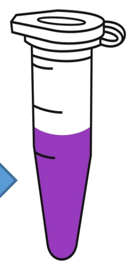




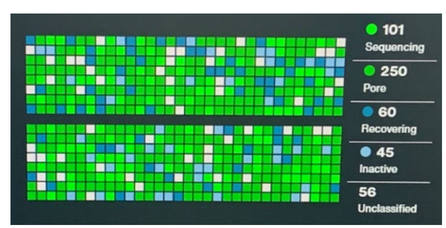
Phytopathology: in review

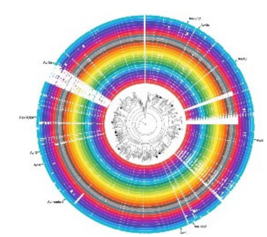
Developing a “Marple's” platform using targeted amplicon sequencing to monitor emerging traits of new lineages and changes within US-23 lineage

- US - 23 clonal lineage widespread and sensitive to mefenoxan.
- All US 23 are not the same- differ in virulence and host specificity.
- Need better phenotypic markers and genotyping to guide management
- Resistance breaking strains of US-23 *P. infestans can* overcome Ph 2+3 genes in tomato
- Species and lineage id
- Fungicide senstivity markers CesA3 (mandipropamid resistance, Blum et al. 2010); OSBP (oxathiapiprolin resistance, Mboup et al. 2021)
- Key effectors (e.g. AVR3a/b, PexRD24 plus more), other traits?

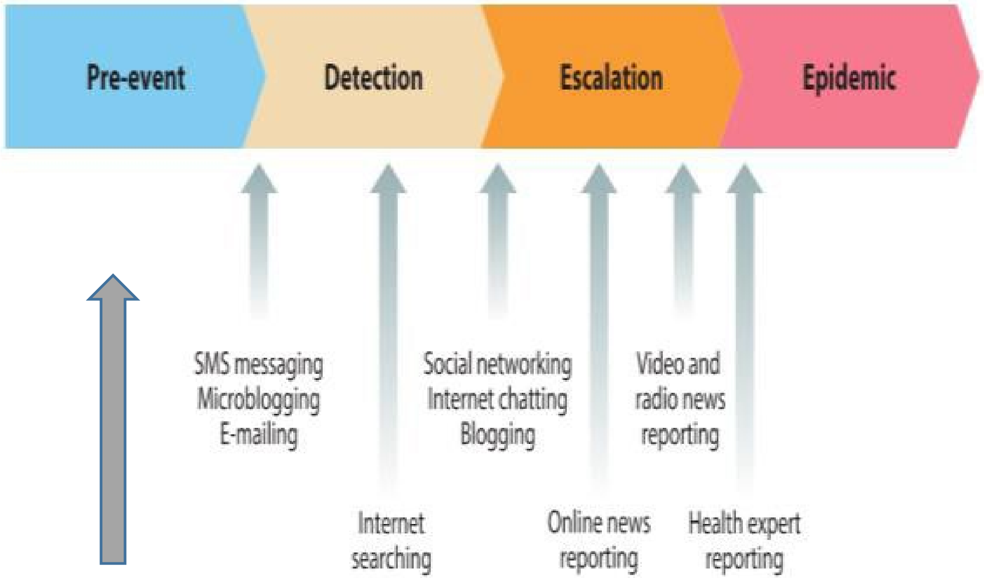


Fast extraction, PCR targets, MinIon sequence, analyze results, incorporate into T BAS





Disease Surveillance



Can we detect phenotypic traits early before symptoms occur ?

Tomato Disease Diagnostics LAMP assays for all

- 1.85 billion dollar industry
- NC is ranked 6th in nation in production with over 4000 acres grown
- Foliar diseases require 15 or more fungicide or pesticide applications

Early Blight- *Alternaria linariae*

Bacterial spot – *Xanthomonas perforans*

Amanda Saville

Tatsiana Shymanovich

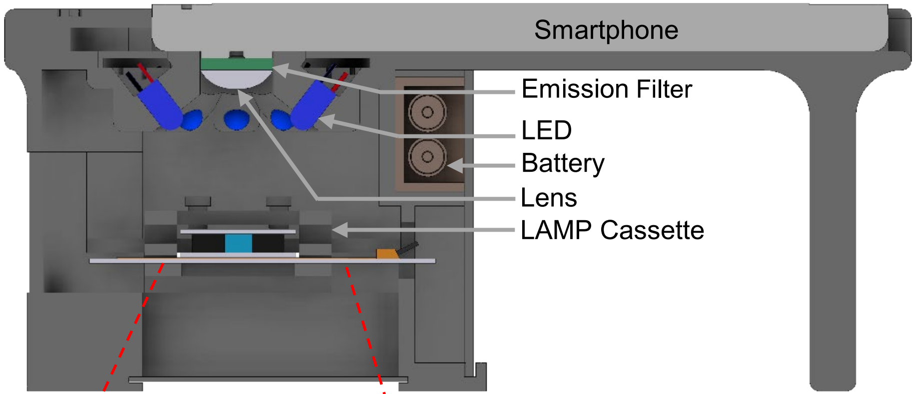
Late Blight- *Phytophthora infestans*

Tomato spotted wilt virus- TSWV

Deploying and testing LyoBead and smartphone LAMP assays for rapid in field detection of specific lineages of *Phytophthora infestans*.

Q. Wei Rajesh Paul

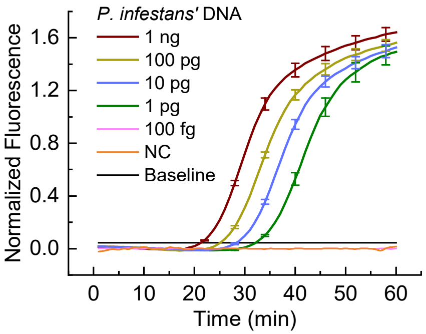
Smartphone Fluorescent Reader

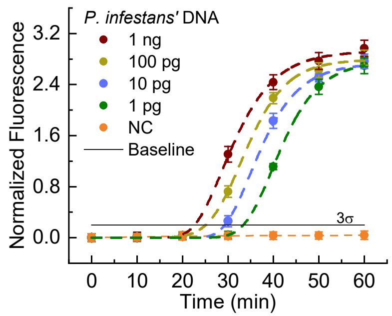


Benchtop real-time thermocycler

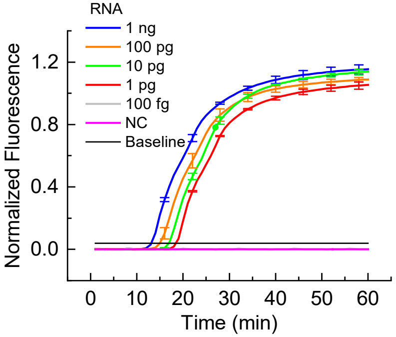
Smartphone-based Platform

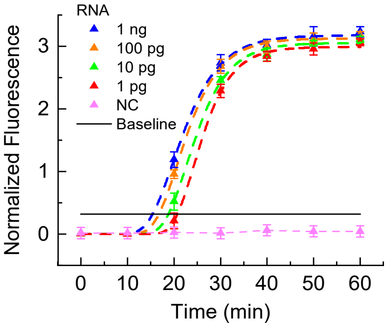
P. infestans

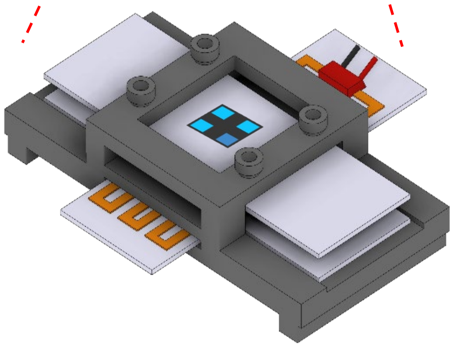




TSWV







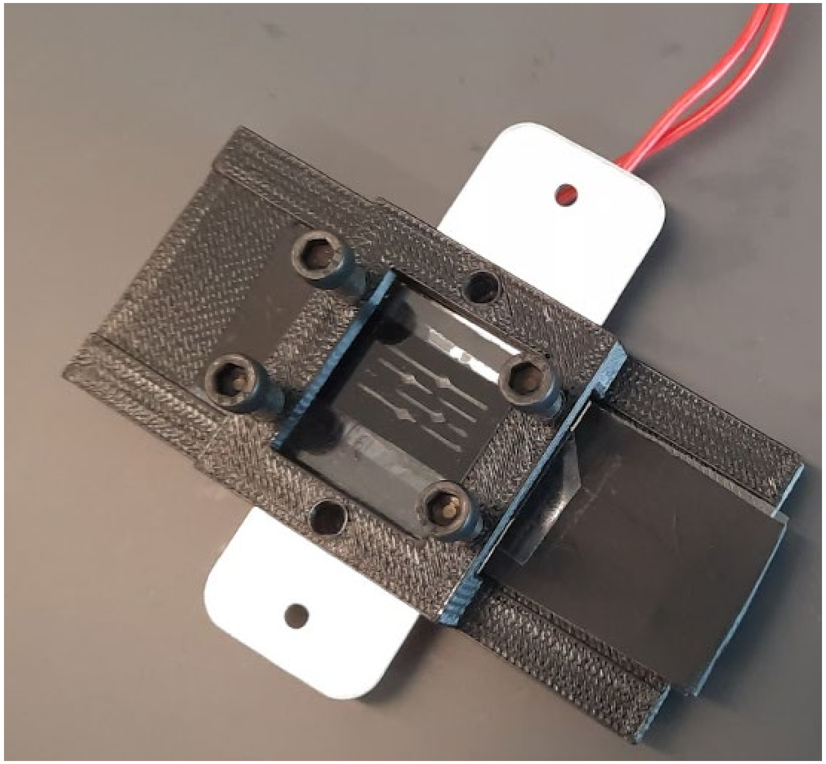
Heated sample cassette

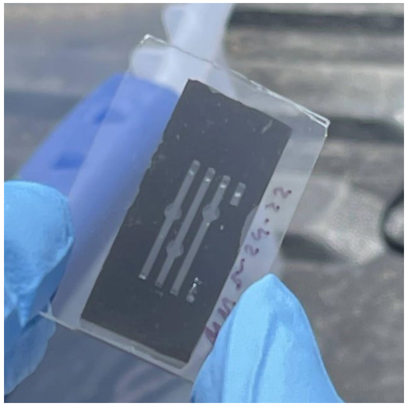
Patterned MN Patch

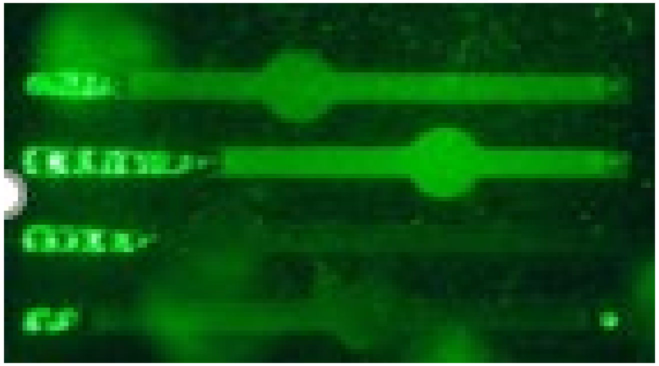
Ristaino et al., 2020. Comparison of LAMP, real-time and Digital PCR for detection of *Phytophthora infestans*. Plant Disease 104:708-716.

Paul, R. et al 2020. Integrated Microneedle-Smartphone Nucleic Acid Amplification Platform for In-Field Diagnosis of Plant Diseases. Biosensors and Bioelectronics 187:113312

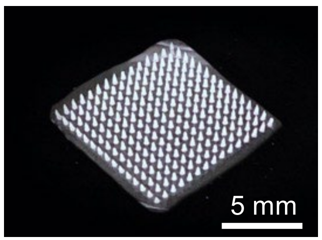
Version 2.0 Smart phone microfluidic LAMP cassette

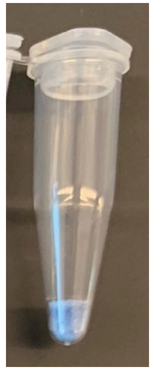


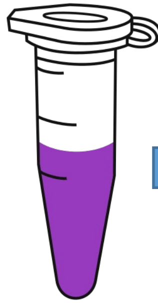




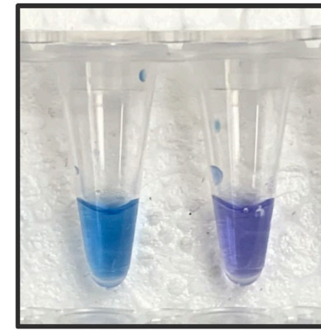
P. infestans LyoBead LAMP



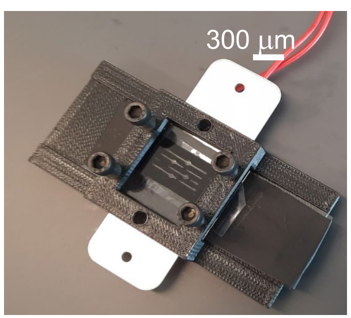


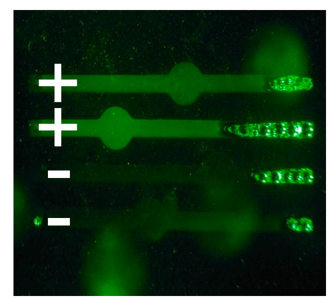


MN extractions followed by 20 min LAMP with lyobeads



Run reaction on AgDia heat block

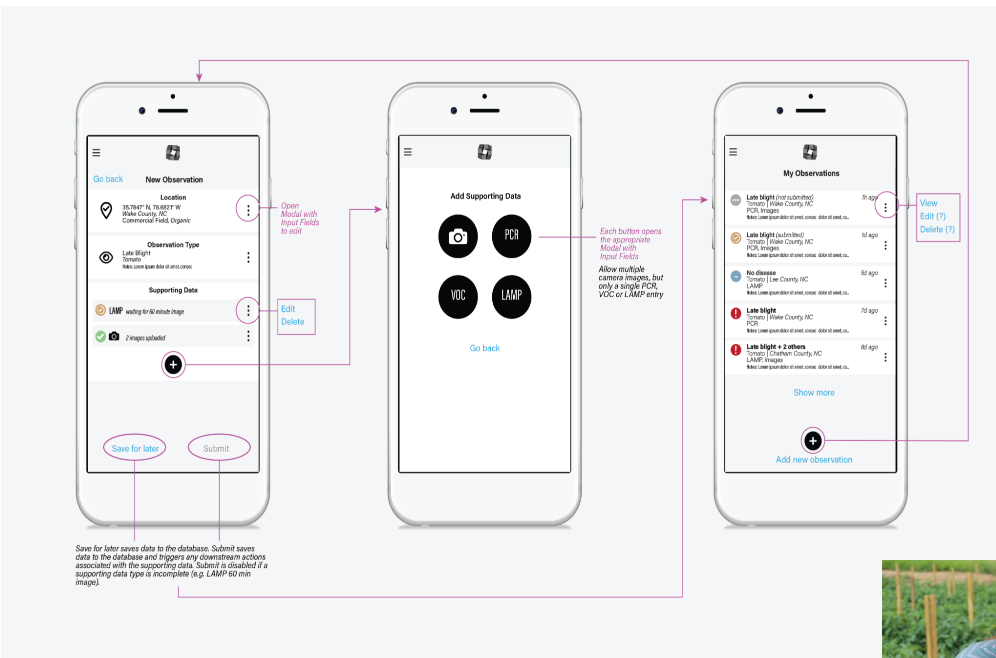


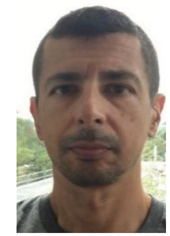


All *Phytophthora*
P. infestans
Healthy leaf
NTC

Run reaction on heated smartphone chip
Visualize a fluorescence change

Link LAMP data collected in the field with PaDB via a web portal





Chris Jones John Polo

-Image analysis software being trained to read the LAMP cassette results
--Linking sensor data to PaDB platform

Issues to resolve

How do we scale detection technology?
What targets do we monitor?-fungicide resistance, resistance breaking strains, host specific lineages, aggressiveness traits

Summary of what you learned today

- USA Blight and has now transitioned into the Plant Aid Database (PaDB).
- Developed a global T BAS tool for Emerging *Phytophthora's* and SSR phylogeny and querying system to identify emerging *P. infestans* lineages
- Incorporating population genomics into forecasting systems to track spread of *P infestans* and understand evolution of 1c clade
- Evolution of pathogen effectome in response to host R genes from 100 year of late blight evolution
- Developing a "Marple's" platform using targeted amplicon sequencing to monitor emerging traits of new lineages and changes within US-23 lineage
- Deploying and testing rapid field detection methods using LAMP assays for *Phytophthora infestans.*

USDA NIFA
United States Department of Agriculture
National Institute of Food and Agriculture

USDA Animal and Plant Health Inspection Service

KENAN INSTITUTE
ENGINEERING, TECHNOLOGY & SCIENCE
NC STATE UNIVERSITY

NC STATE UNIVERSITY GRIP4PSI –NC State Office of Research and Innovation

Plant Science Initiative, NC State

BASF
The Chemical Company

NSF

J. Ristaino's laboratory website
http://ristainolab.cals.ncsu.edu//

USAID
FROM THE AMERICAN PEOPLE

NC STATE UNIVERSITY

FULBRIGHT

Dole

THE ROCKEFELLER FOUNDATION