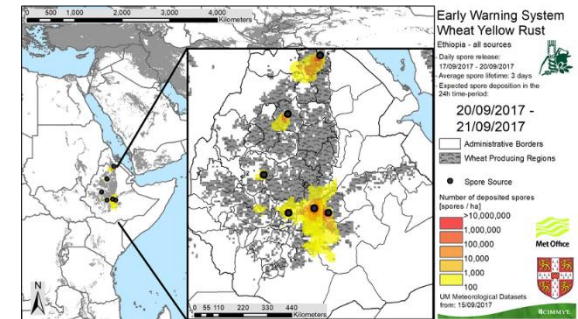# Molecular Surveillance Tools + Early Warning Systems
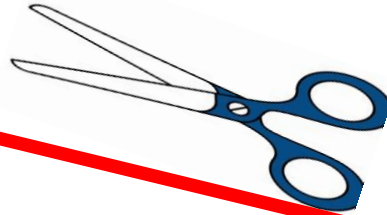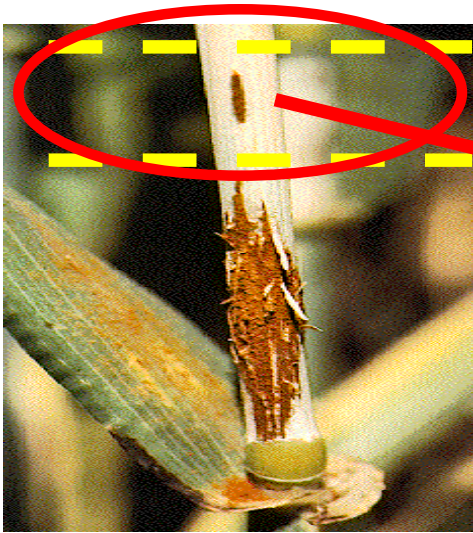
Dave Hodson

CIMMYT-Ethiopia

d.Hodson@cigar.org

Kenya Njoro Stem Rust
Training Oct 2017

Samples: Molecular diagnostics – additional option

Race Analysis

- Provided most information
- Issues:
  - Shipping live samples
  - Lab capacity

Molecular Diagnostics

- Increasingly valuable option
- SNP markers: Rapid diagnostic for Ug99 race group
- Expanding to other important races
- Permitting genotype identification + population insights

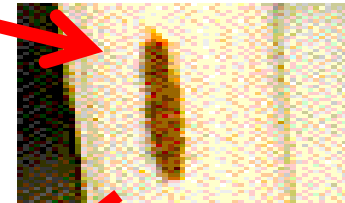Credit: Dr Les Szabo, USDA-ARS CDL

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Stem Rust: DNA Sampling Protocol

**[NB: Clean scissors + hands with Ethanol after each sample]**

1. Select a single, large, isolated pustule. Cut the stem either side of the pustule. [NB: **Only 1 pustule per tube**]
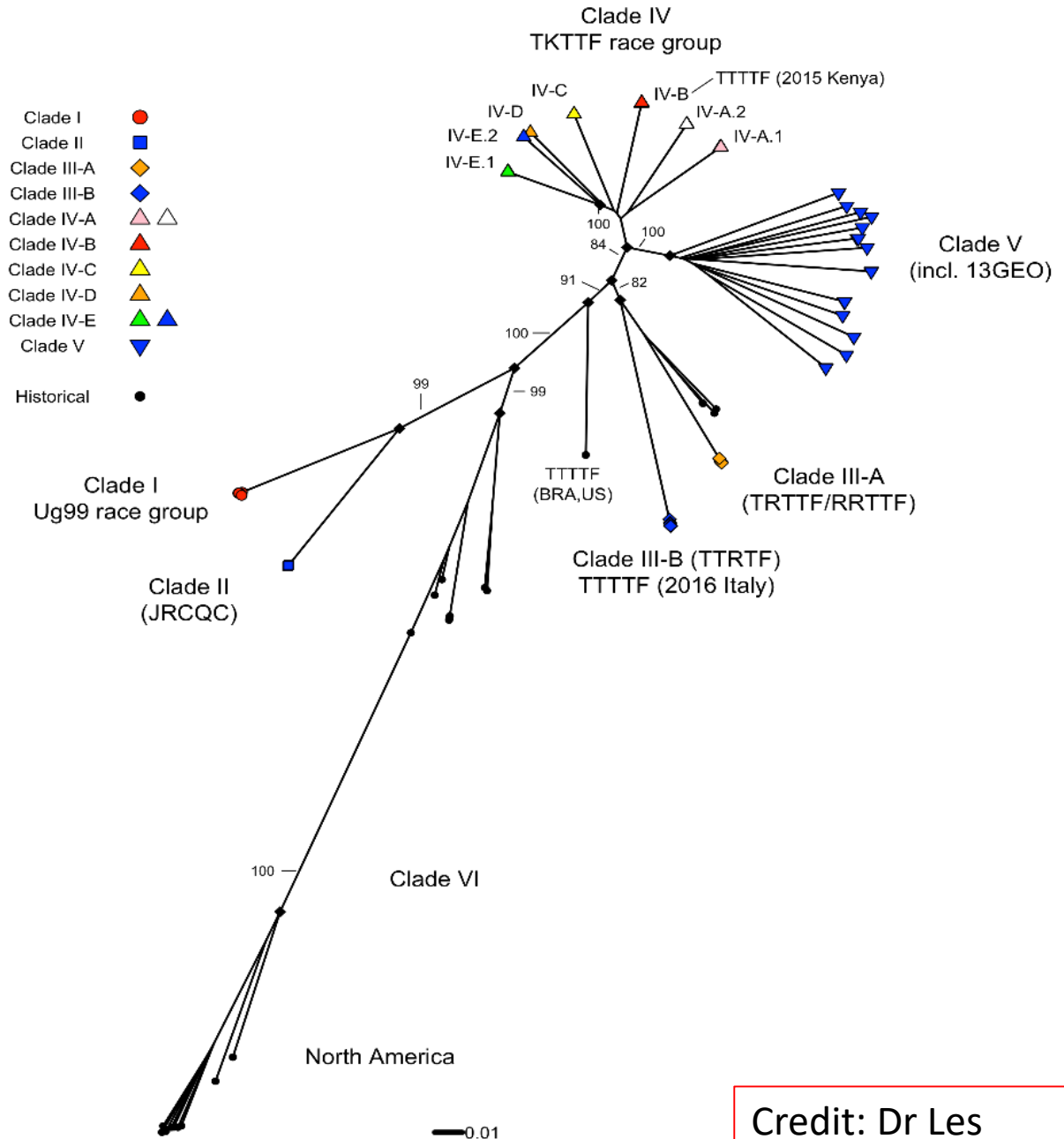
2. Remove the core stem tissue

3. Place pustule (and sheath tissue) into a cryovial. Add 70% Ethanol (or absolute alcohol) to ¾ fill the cryovial (ensure pustule is completely covered). Label tube + record unique code on survey form
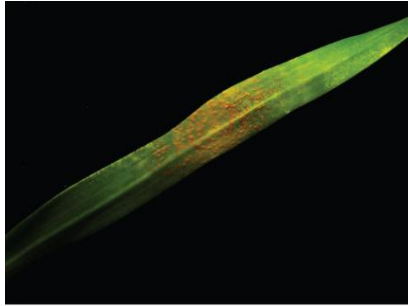
+
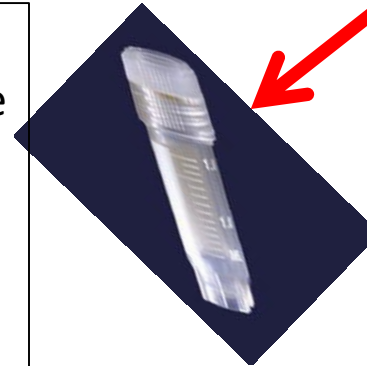
4. Store in ethanol for 7 days. Decant, air dry (with tube cap off) for 12-24 hours., seal the tube, place in zip lock bag and send via courier to Cereals Disease Lab, Minnesota
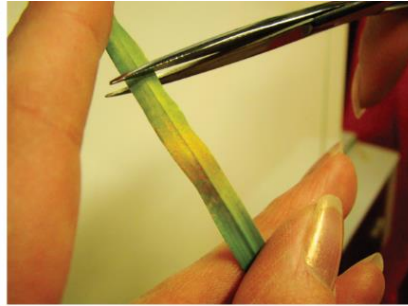
New Te
and po[

- **Molecular diagn**
  assay for wheat [
  (field collected s

  - Stage 1: Ug9[
  - Stage 2: Diag[

- **Population gene**
  genotyping SNP [
  genetics and diag[

  - 1. PgtSNP 1.5[
    - - 50% geno[
  - 2. PgtSNP 3.0[
    - - 98% geno[

Clade IV
TKTTF race group

Clade I
Clade II
Clade III-A
Clade III-B
Clade IV-A
Clade IV-B
Clade IV-C
Clade IV-D
Clade IV-E
Clade V

Historical

IV-C
IV-D
IV-E.2
IV-E.1
IV-B — TTTTF (2015 Kenya)
IV-A.2
IV-A.1

Clade V
(incl. 13GEO)

100
84
100
91
82
100
99
99

Clade I
Ug99 race group

Clade II
(JRCQC)

TTTTF
(BRA,US)

Clade III-A
(TRTTF/RRTTF)

Clade III-B (TTRTF)
TTTTF (2016 Italy)

100

Clade VI

North America

0.01

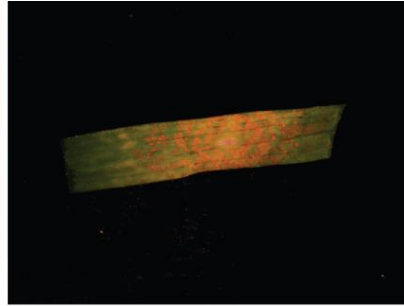United States Dep[
Agricultural Rese[
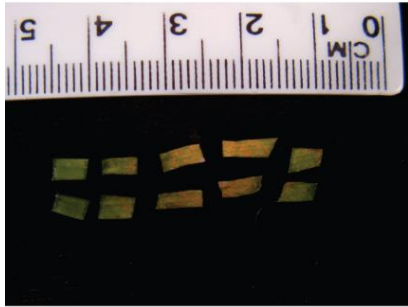
Stripe Rust – RNA Samples

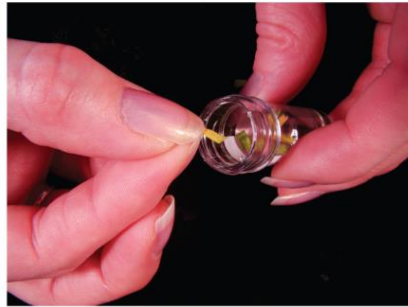**1.** Select a leaf with clear yellow rust pustules.

2. Cut out a region close to the infection symptom (pustules) area.

3. Trim the leaf fragment to the pustule region.

4. Cut the large pustule-containing fragment into small segments.

**5.** Place the small segments into the supplied tube containing RNAlater solution. Briefly shake so segments are soaked in the solution. Ensure lid is tightly fastened.

6. Label tube with: Name, Date of collection and Location. Also complete the enclosed sampling form with full details.

- Credit: Dr Diane Saunders (JIC)
- Contact CIMMYT for Tubes + shipping help

Genome Biology 2015 **16**:23
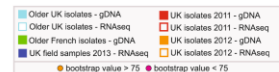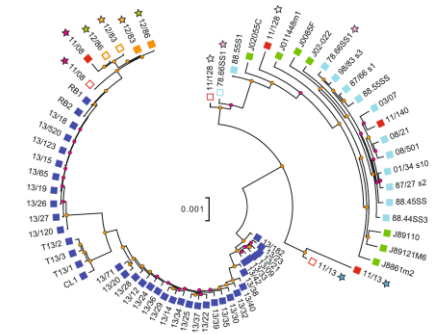
Field Pathogenomics

- a robust and rapid method

- based on RNA sequencing directly from infected host samples to gain insight into emerging pathogen populations.

- Field pathogenomics should be applicable to surveillance of many pathogens
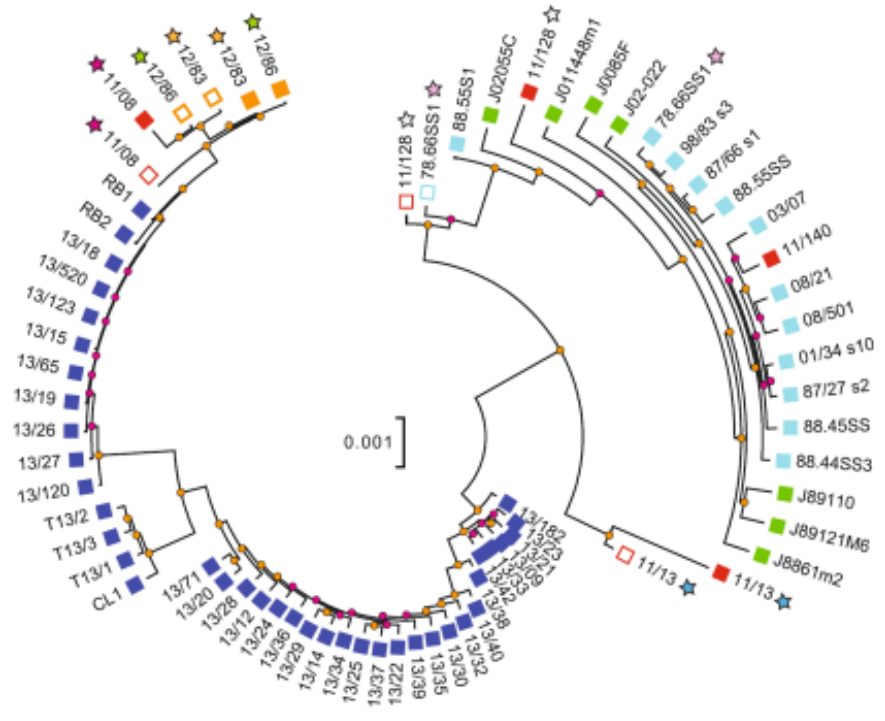
The Pathogen:
Puccinia Striiformis f.sp *Tritici*
"a shifty, changing, constantly evolving enemy"

The Technology: MinION mobile sequencing + Field Pathogenomics

Welcome to the Future of Pathogen Diagnostics

Real-time, Mobile Wheat Rust Diagnostics in Ethiopia

Dave Hodson (CIMMYT-Ethiopia)
d.hodson@cigar.org
Diane Saunders (John Innes Centre, UK)
Diane.Saunders@jic.ac.uk
Endale Gebre (EIAR, Ethiopia)
endale_gebre@yahoo.com

CIMMYT MR
International Maize and Wheat Improvement Center

John Innes Centre
Unlocking Nature's Diversity

Problem:

- Every season in the dark with the pathogen
  - What are we dealing with? (New race? Something Highly Virulent?)

- Conventional diagnostics
  - Slow (months to year for results)
  - Very few samples + highly specialised labs

- Lack of early warning : Epidemic on farmers fields

Solution: Diagnostics as fast as the pathogen is changing / moving

- Real time, data driven diagnostics (MinION + Field Pathogenomics)
  - What we have, where

- Early warning - Informed, fast, optimal control

- Used for Ebola / Zika – in the field
- Now it's time for crop pathogens!

Innovation – Mobile, Pathogen diagnostics real-time

Usability – Low $, in-country deployment

Data Driven Diagnostics

New!

Early Warning Alert + Control

Impact

Data Integration

Scalability
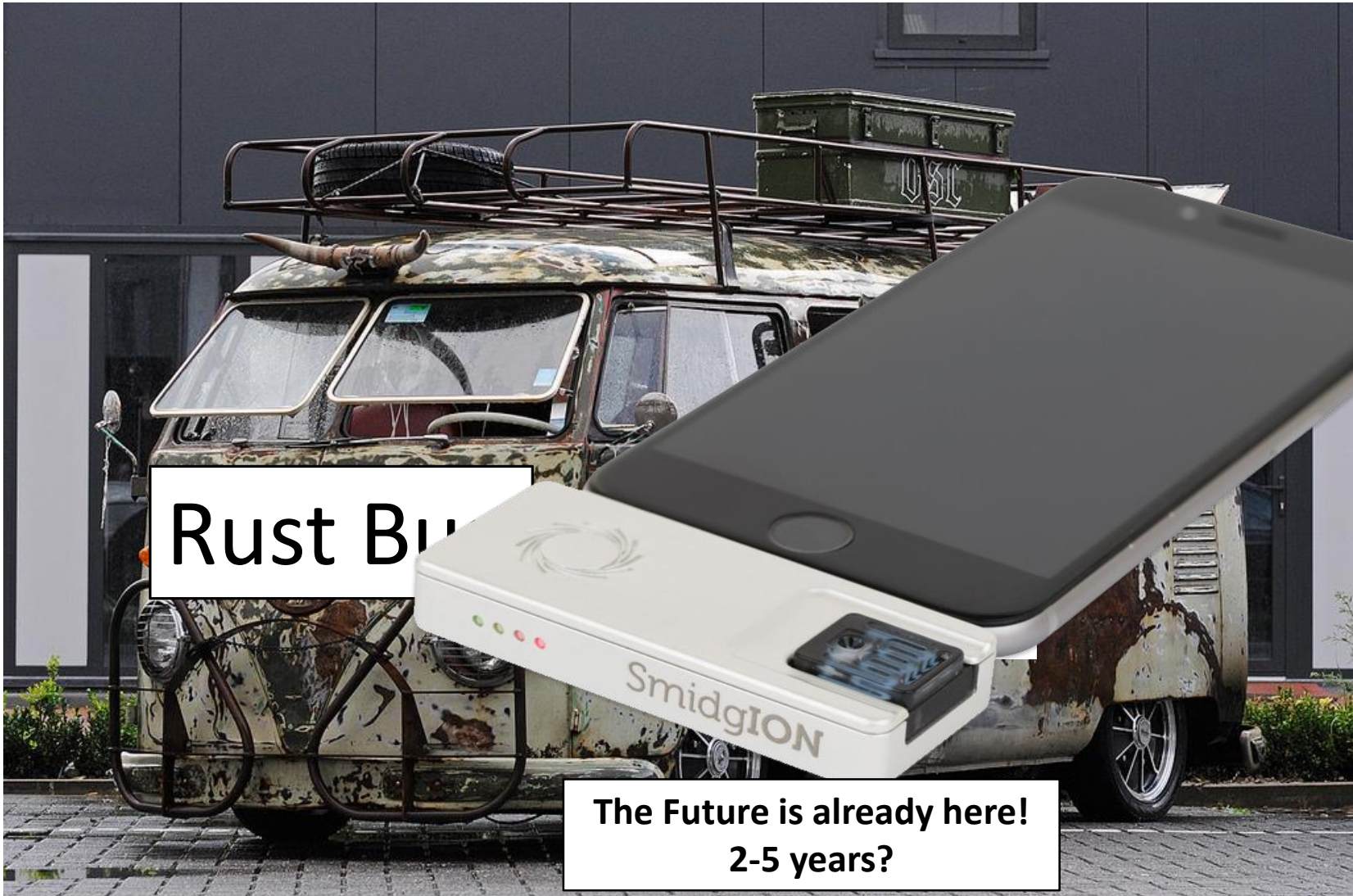
Other pathogens, countries, crops

Risk Forecast

Mobile Pathogen Diagnostics Platform
(no demo available!)

Rust Bus

SmidgION

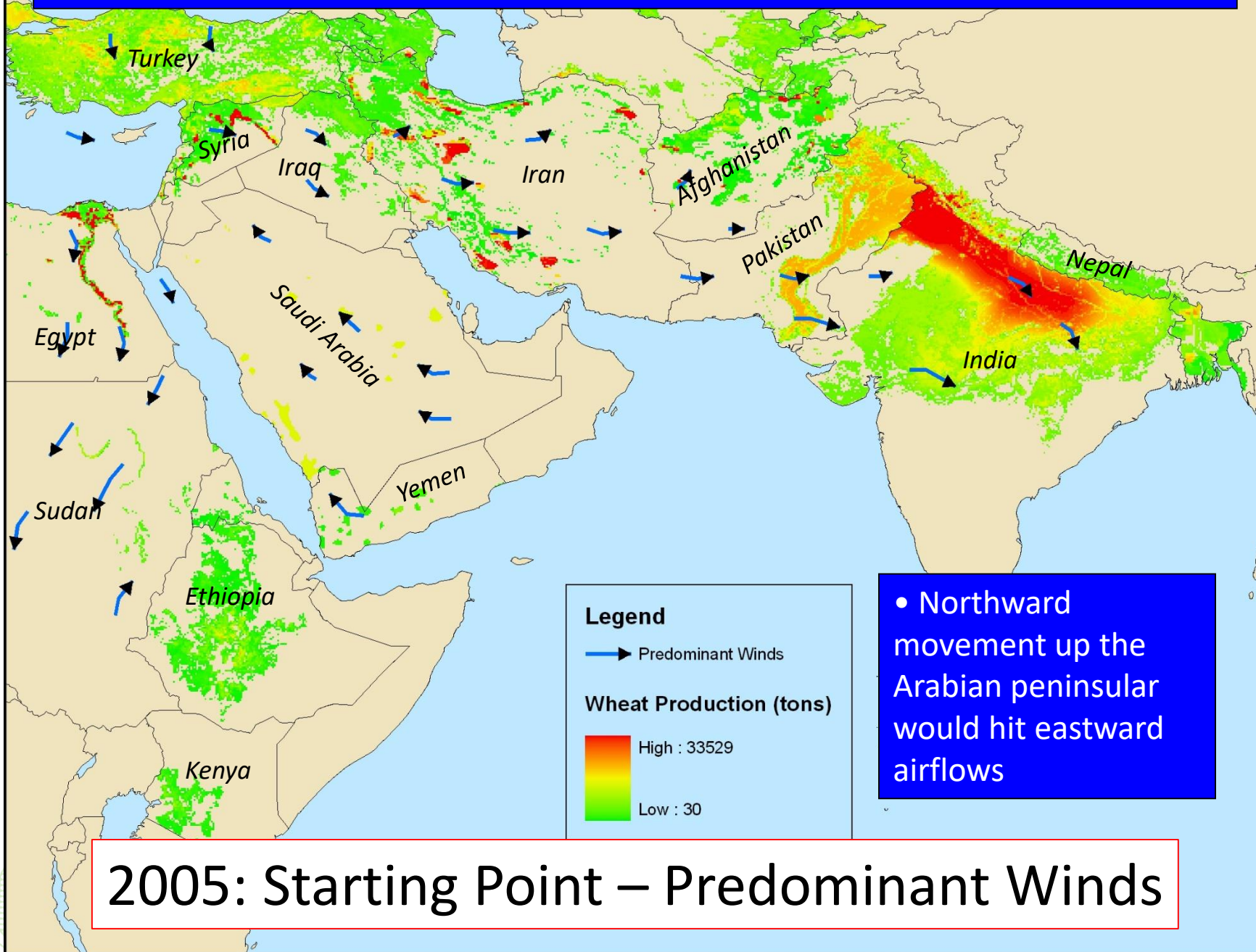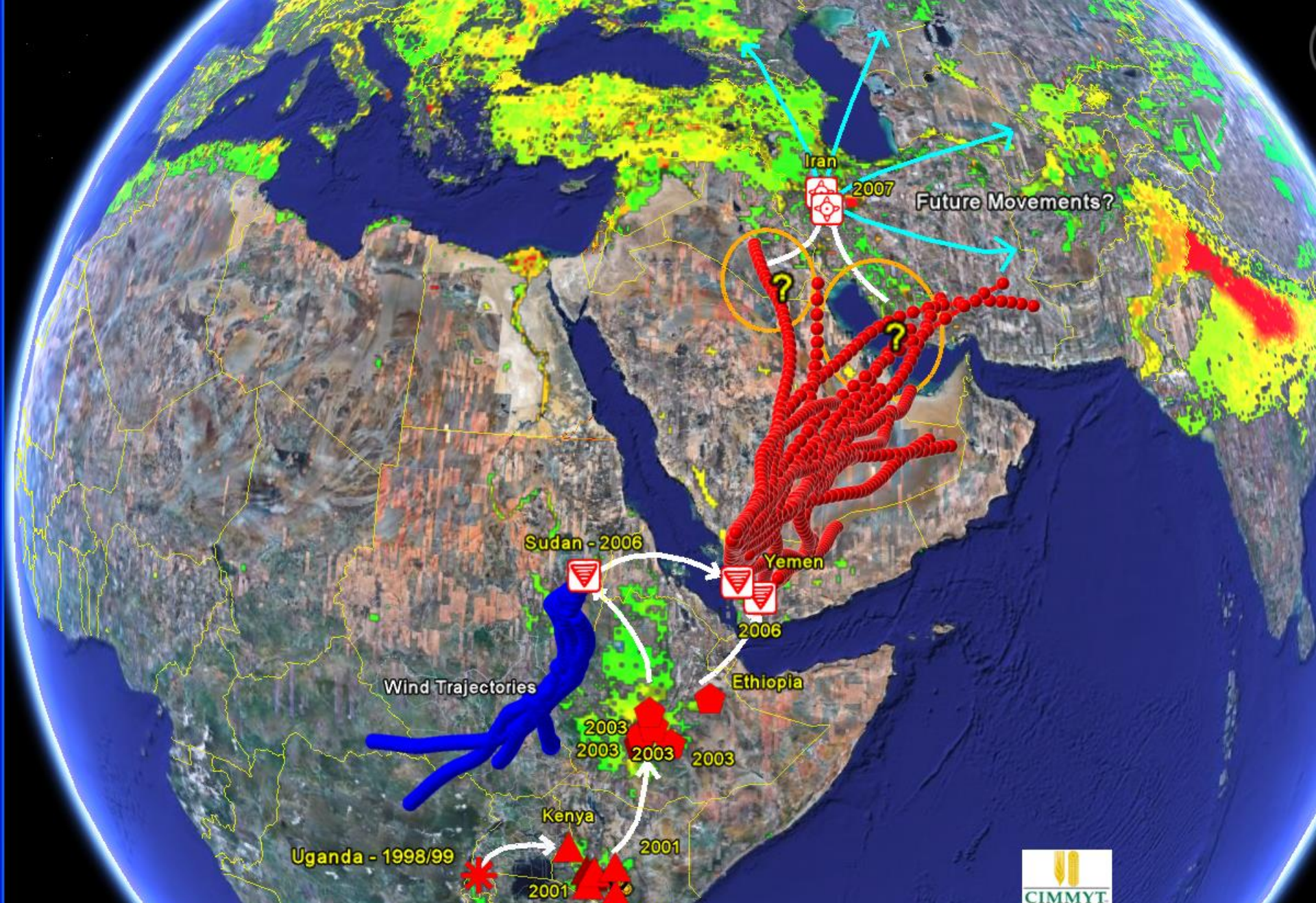**The Future is already here!**
2-5 years?

Gone With the Wind

Emerging Early Warning Systems

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Where and Why Might UG99 Spread? – 3. Predominant Regional Winds
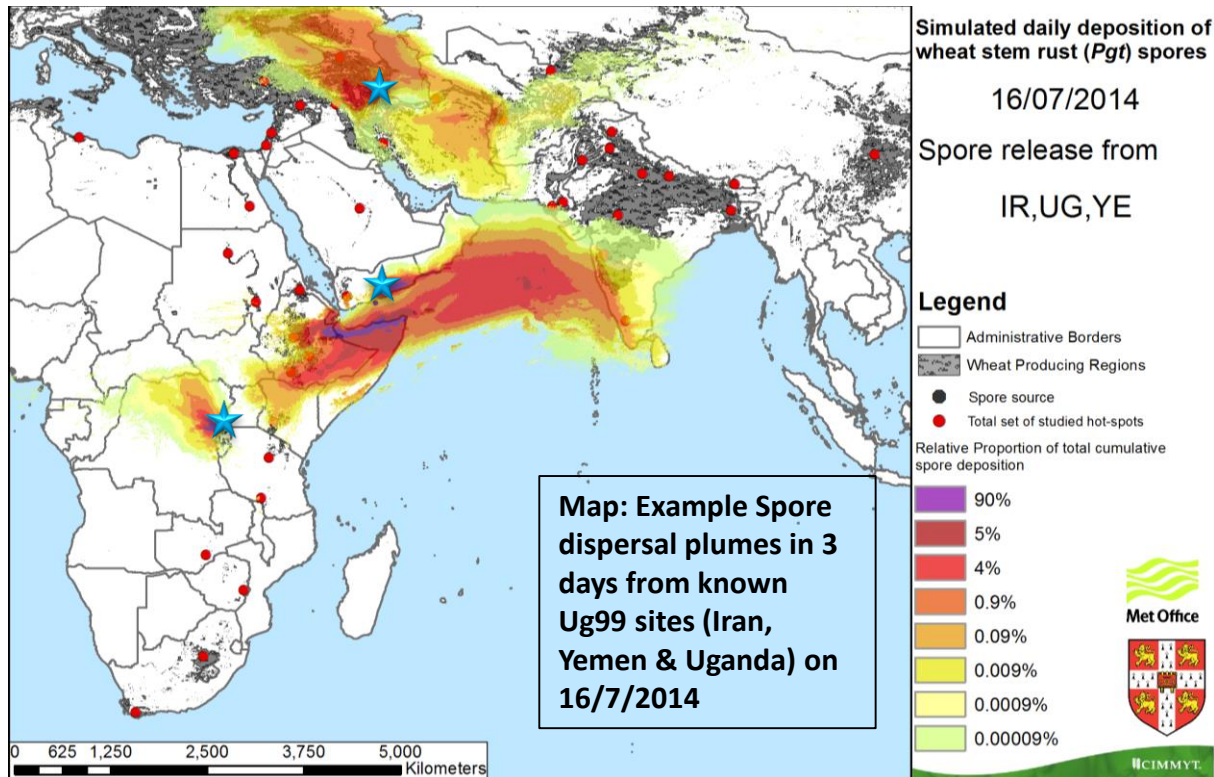
2005: Starting Point – Predominant Winds

2007: HYSPLIT – Trajectory Model

Stem Rust Spore Dispersal Modelling - Emerging early warning systems

⭐ Ug99 Sites – Simulated spore release

Simulated daily deposition of wheat stem rust (*Pgt*) spores

16/07/2014

Spore release from

IR,UG,YE

Legend

☐ Administrative Borders
▨ Wheat Producing Regions
● Spore source
● Total set of studied hot-spots

Relative Proportion of total cumulative spore deposition

	90%
	5%
	4%
	0.9%
	0.09%
	0.009%
	0.0009%
	0.00009%

Met Office

Map: Example Spore dispersal plumes in 3 days from known Ug99 sites (Iran, Yemen & Uganda) on 16/7/2014

0 625 1,250   2,500   3,750   5,000
                                    Kilometers

Credit: M. Meyer, Cambridge University

UNIVERSITY OF CAMBRIDGE   Met Office

Pathogens without Borders

- Rust spores capable of travelling 1000's of Km in a few days
- Using advanced models: Quantified the probability and seasonality of migration between countries and regions
- Developing rust early warning / forecast systems (E.g., In Ethiopia forecast risk mapping)

2014: Advanced NAME Dispersal Model

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First Quantitative Assessment of Airborne Migration routes

- Finely resolved meteorological datasets in a mechanistic modelling framework (3 hourly time step, 17km2 grid)

- High-performance computational resources (20TB data, UK Met Office supercomputer)

- First quantitative estimates of when, how often, and how much viable spore material is dispersed from infected areas to different regions and continents.
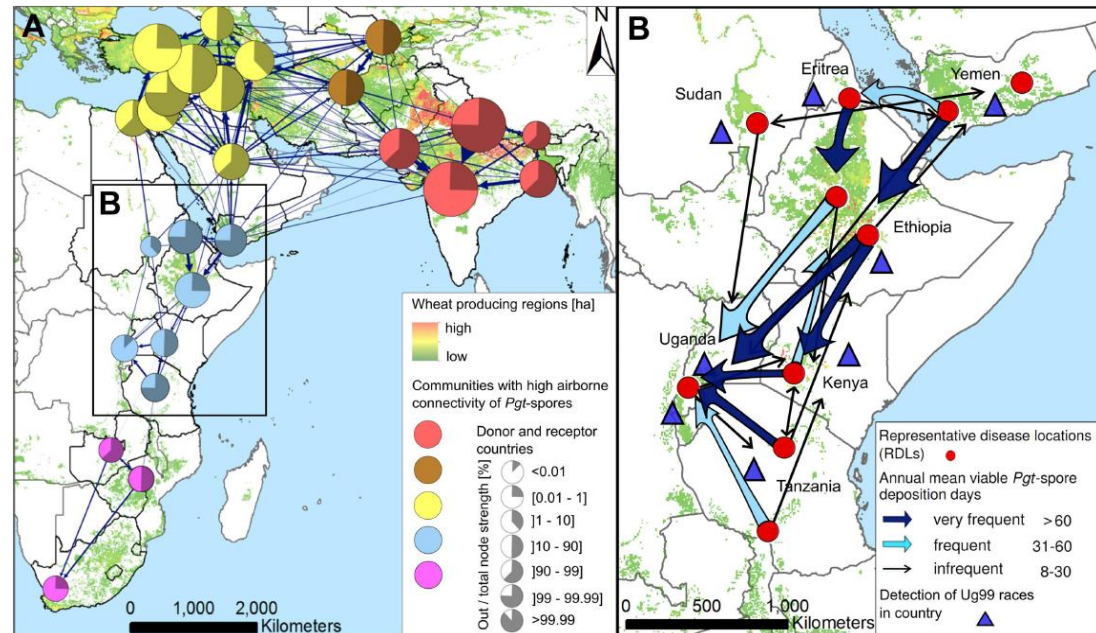
Meyer et al 2017 Nature Plants

Caution for South Asia??

- Increasing stem rust survey reports – Azerbaijan, Iraq, NW Iran [**NB: NO large epidemics reported + timing end April/early May**]

- Most likely – TKTTF / TTTTF variants?

- Caution with SrTmp, Sr25 varieties [Muqawim-09, Koshan-09 in Afghanistan, others???] + Durums

- Dispersal models (Meyer et al 2017):

  - **very small / small outbreaks in Iran = no direct transport from Iran to Pakistan and India**.

  - BUT - scenario of **moderate to large outbreaks in Iran** ($>10^3$ ha, with >15% severity and incidence) = rare to infrequent direct transport from Iran to Pakistan, and **frequent (31-60 days per year) to very frequent (>60 days per year) stepwise transmission first from Iran to Afghanistan, and then further from Afghanistan to the northern plains of Pakistan/India**.

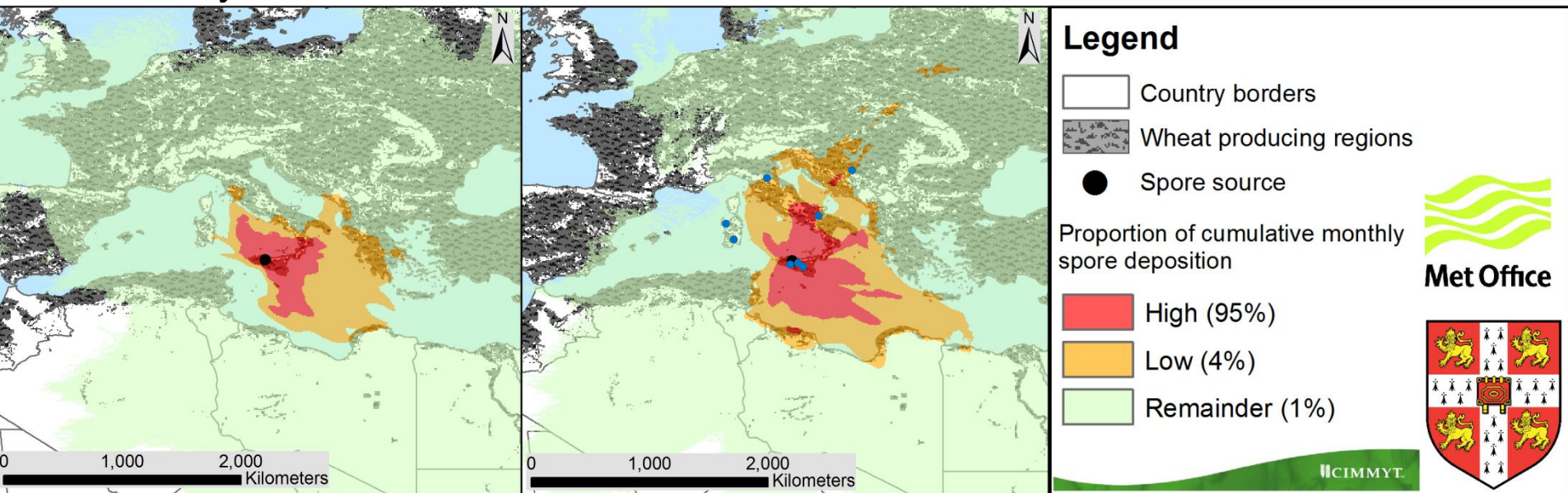Stem Rust survey reports 2016

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Regional Warning: Example

- First Detection race TTTTF Sicily April/May 2016
- Using spore dispersal models – Alert to potential at risk countries
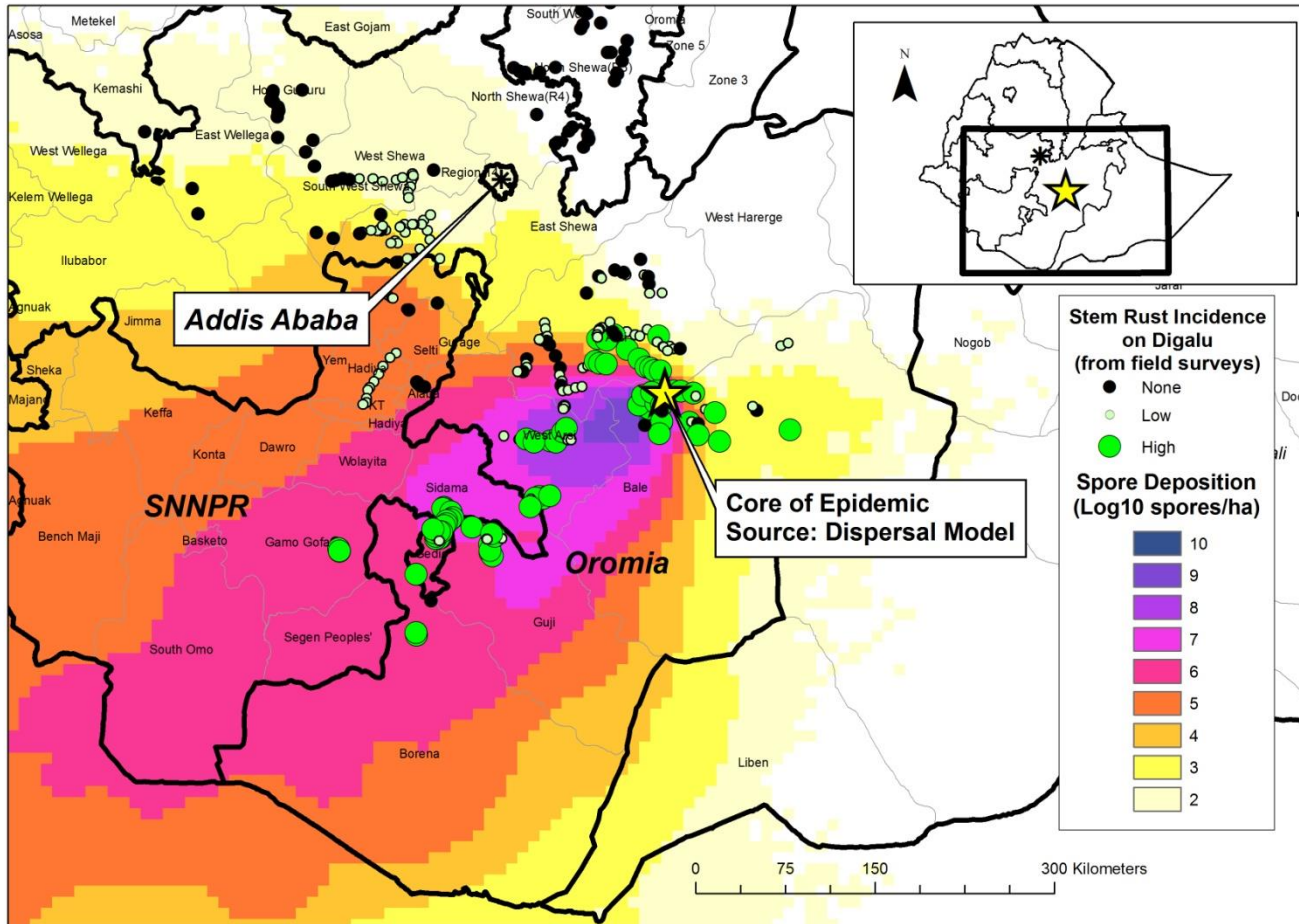
May 2016   June 2016

Legend

- Country borders
- Wheat producing regions
- ● Spore source

Proportion of cumulative monthly spore deposition

- High (95%)
- Low (4%)
- Remainder (1%)

Met Office

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Likely *Pgt*-spore dispersal from the Sicily site (preliminary assessment)

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Emerging Early Warning Systems

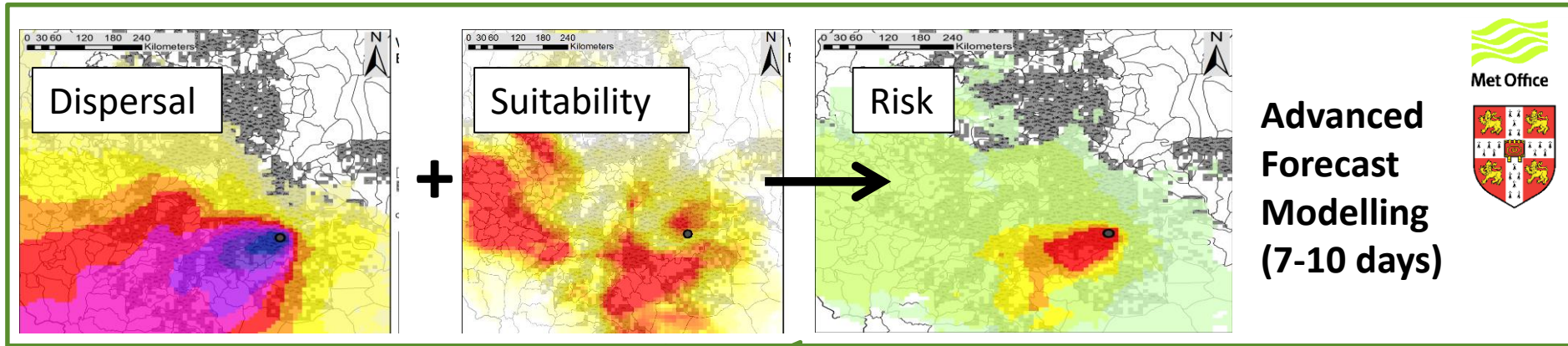Ethiopia – Spore dispersal Nov 2013 (Race TKTTF epidemic)

- Spore dispersal / deposition models (close match to observed field data)

- Quantified probability of spore migration pathways at regional scales

Credit: Cambridge University, UK + UK Met Office (see Olivera et al., 2015)

Early Warning – Framework (Ethiopia)

Dispersal + **Suitability** → **Risk**

Advanced Forecast Modelling (7-10 days)

Met Office

EIAR Central Unit

MoA — Regions — Ext

Control Awareness Scouting

Extension – Crowd source disease information (near real time data)

Field Survey – near real-time data

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Ethiopia 2016: Pilot Early Warning System

- Early field detection of rust (surveys)
- Forecast modelling pipeline established
- Advance warning to government agencies
- 2016 Awareness / preparedness and control ahead of disease in some key areas (based on feedback received)
- Extended system planned for 2017 in Ethiopia. BBSRC project
- If successful transfer to other areas beyond Ethiopia
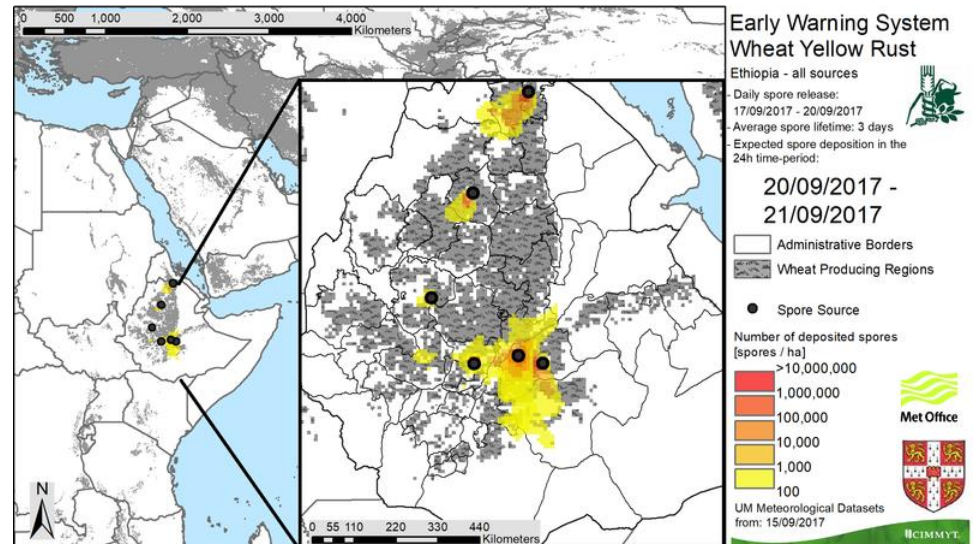
Summary

- Molecular tools giving new insights into pathogen populations
- Options emerging for real-time diagnostics
- Early warning systems developing
  - Regional level
  - In-country, in-season early warning / forecasting

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Thanks to our Donors