Ug99: Challenges of Working with an Emerging Race of the Wheat Stem Rust Pathogen

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Background – Rust for Dummies

Wheat Rusts

Stem rust
Black stem rust
Puccinia graminis
f.sp *tritici*

Stripe rust
Yellow rust
P. striiformis
f.sp. *tritici*

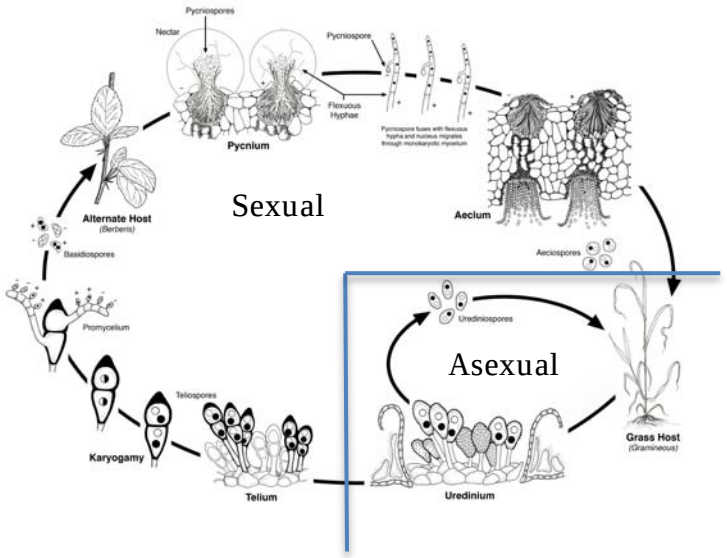
Leaf rust
Brown rust
P. triticina

Wheat Stem Rust

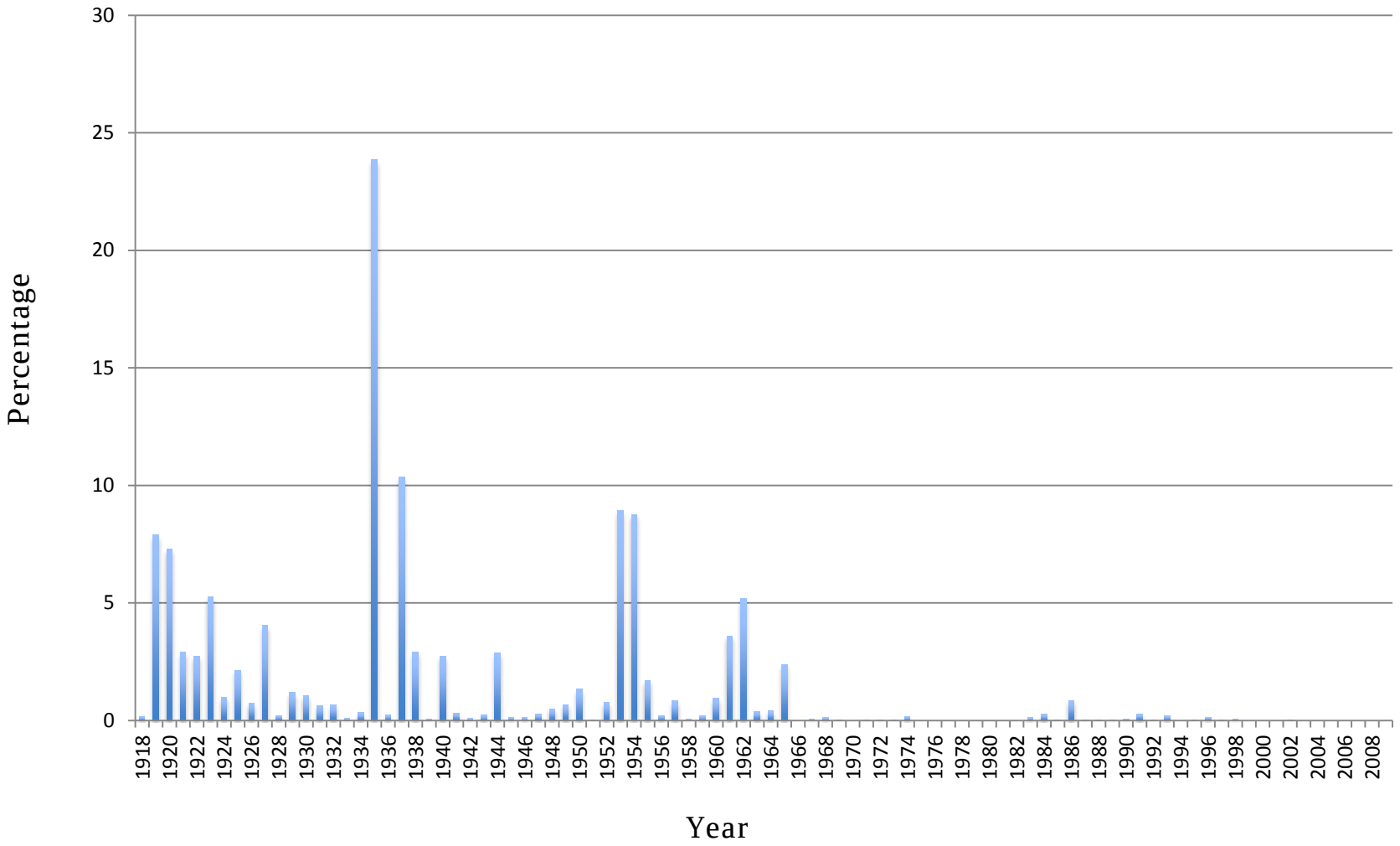
- Historically, the most destructive of the three wheat rust diseases.
- Known to completely destroy a wheat field in less than month.
- Was developed as a biological warfare agent, during the cold war era.

Facts of life

- Obligate biotroph
- Macrocyclic
  - 5- spore stages
- Heteroecious
  - Wheat, barley and grasses
  - *Berberis* and *Mahonia*
- Dikaryotic (n+n)
  - Uredinial stage
    - Asexual



Loss of U.S. Wheat to Stem Rust



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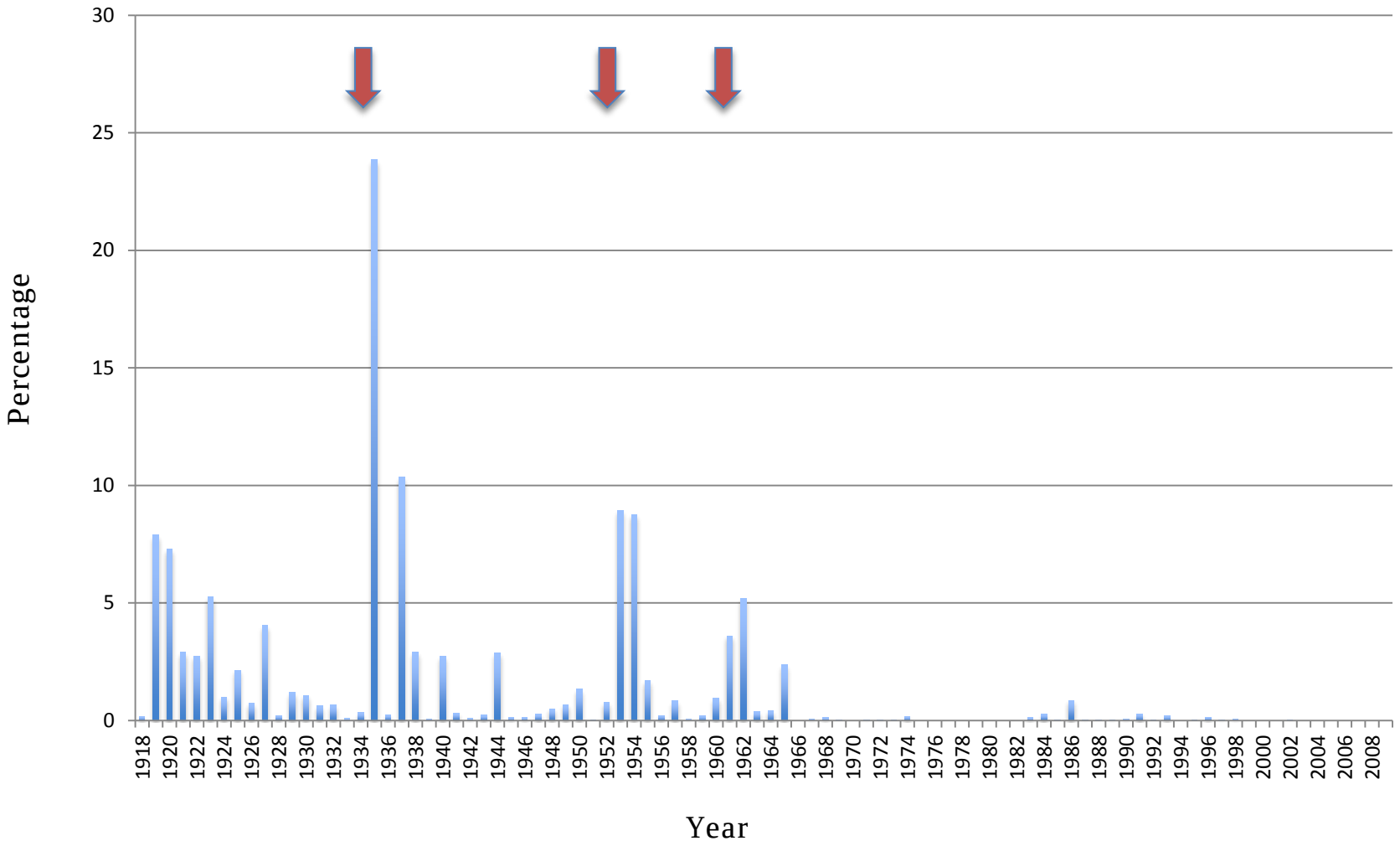

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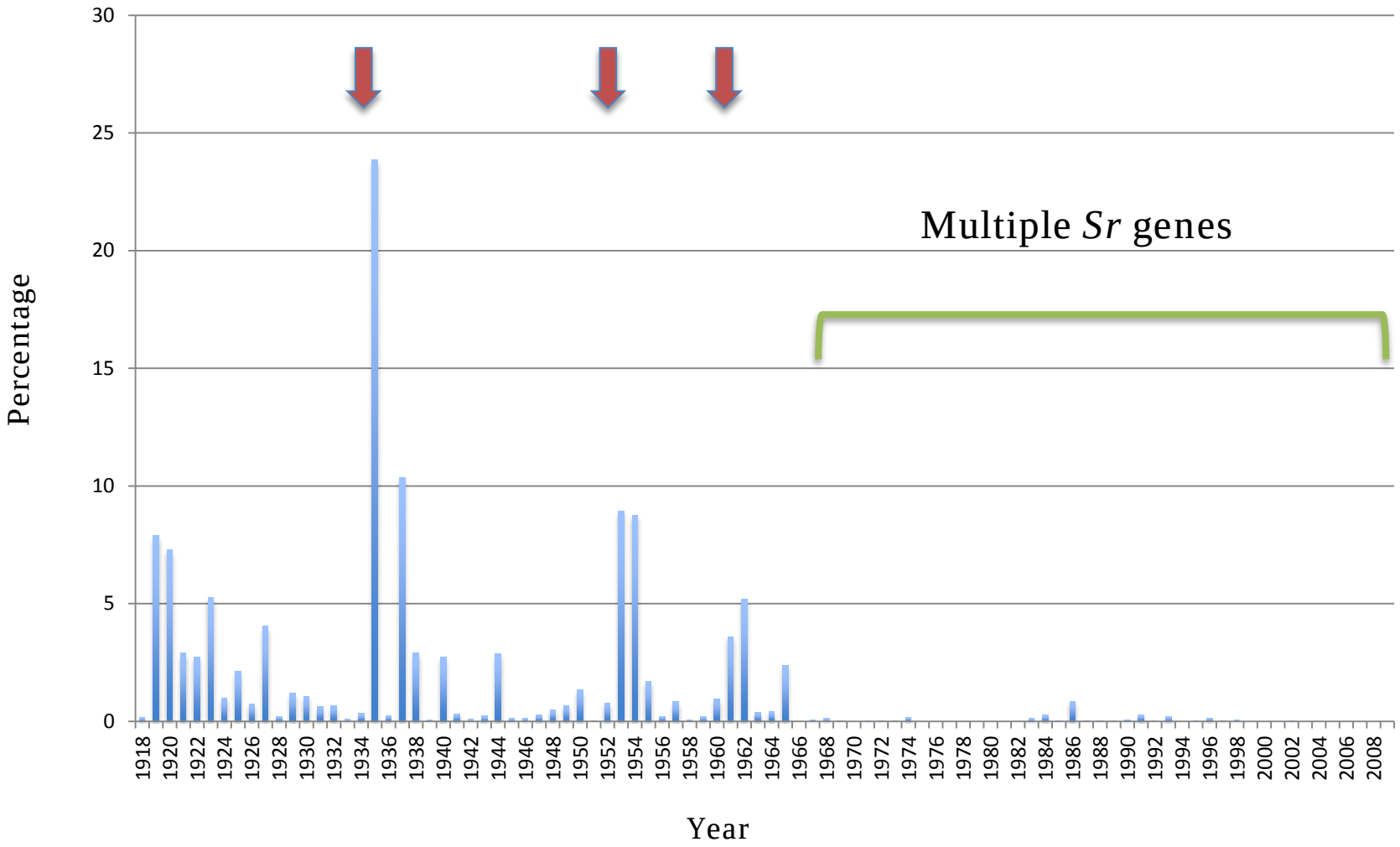
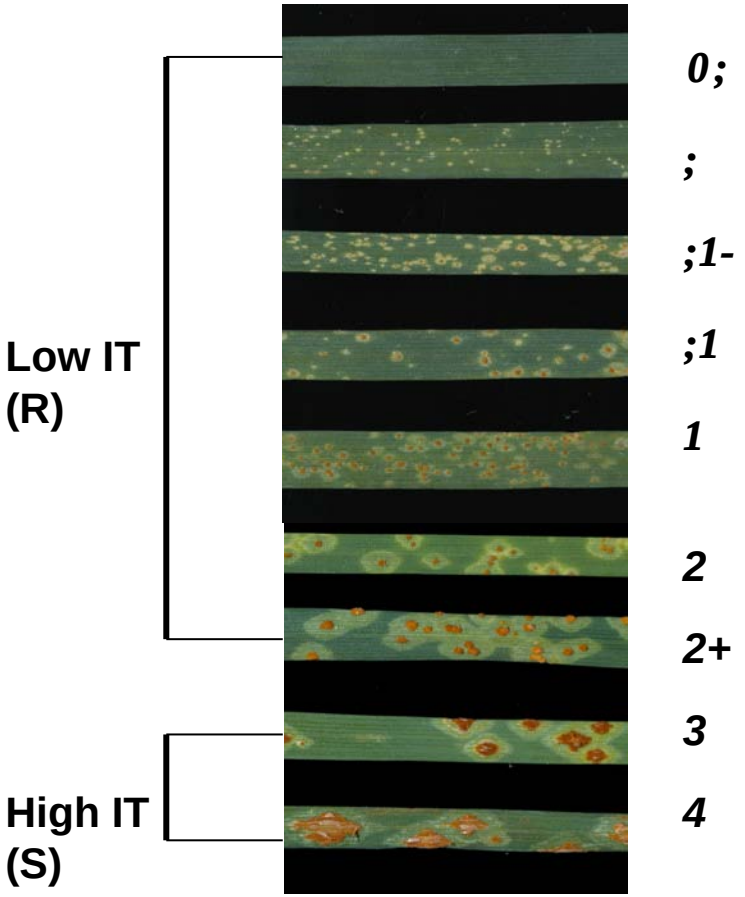


Avirulence/Virulence Phenotypes

- Range of phenotypes
- Characterized using a standard set of differentials.
- Phenotypes are characteristic for specific for R gene/effector gene combinations.



Race Nomenclature

Set	Line	Sr Gene	IT	Race
1	ISr5Ra	*5*	4	
	T.m.deri.	*21*	3+	T
	Verstein	*9e*	4	
	ISr7bRa	*7b*	3	
2	ISr11Ra	*11*	4	
	ISr6Ra	*6*	4	T
	ISr8Ra	*8a*	4	
	CnsSr9g	*9g*	4	
3	W2691SrTt-1	*36*	0	
	W2691Sr9b	*9b*	4	K
	BtSr30Wst	*30*	4	
	Comb. VII	*17+13*	2++	
4	ISr9aRa	*9a*	4	
	ISr9dRa	*9d*	4	S
	W2691Sr10	*10*	4	
	CnsSrTmp	*Tmp*	2+	
5	LcSr24Ag	*24*	2	
	Sr31/LMPG	*31*	4	K
	VPM1 38	*38*	4	
	McNair701	*Mc*	4	

TTKSK

B	R	R	R	R
C	R	R	R	S
D	R	R	S	R
F	R	R	S	S
G	R	S	R	R
H	R	S	R	S
J	R	S	S	R
K	R	S	S	S
L	S	R	R	R
M	S	R	R	S
N	S	R	S	R
P	S	R	S	S
Q	S	S	R	R
R	S	S	R	S
S	S	S	S	R
T	S	S	S	S

Ug99

What is Ug99?

- New highly virulent race of *Puccinia graminis* f. sp. *tritici* (*Pgt*).
- First found in Uganda in 1998 and characterized in 1999.

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- First race of *Pgt* with virulence to wheat stem rust resistance gene *Sr31*.
- Race designation: TTKSK

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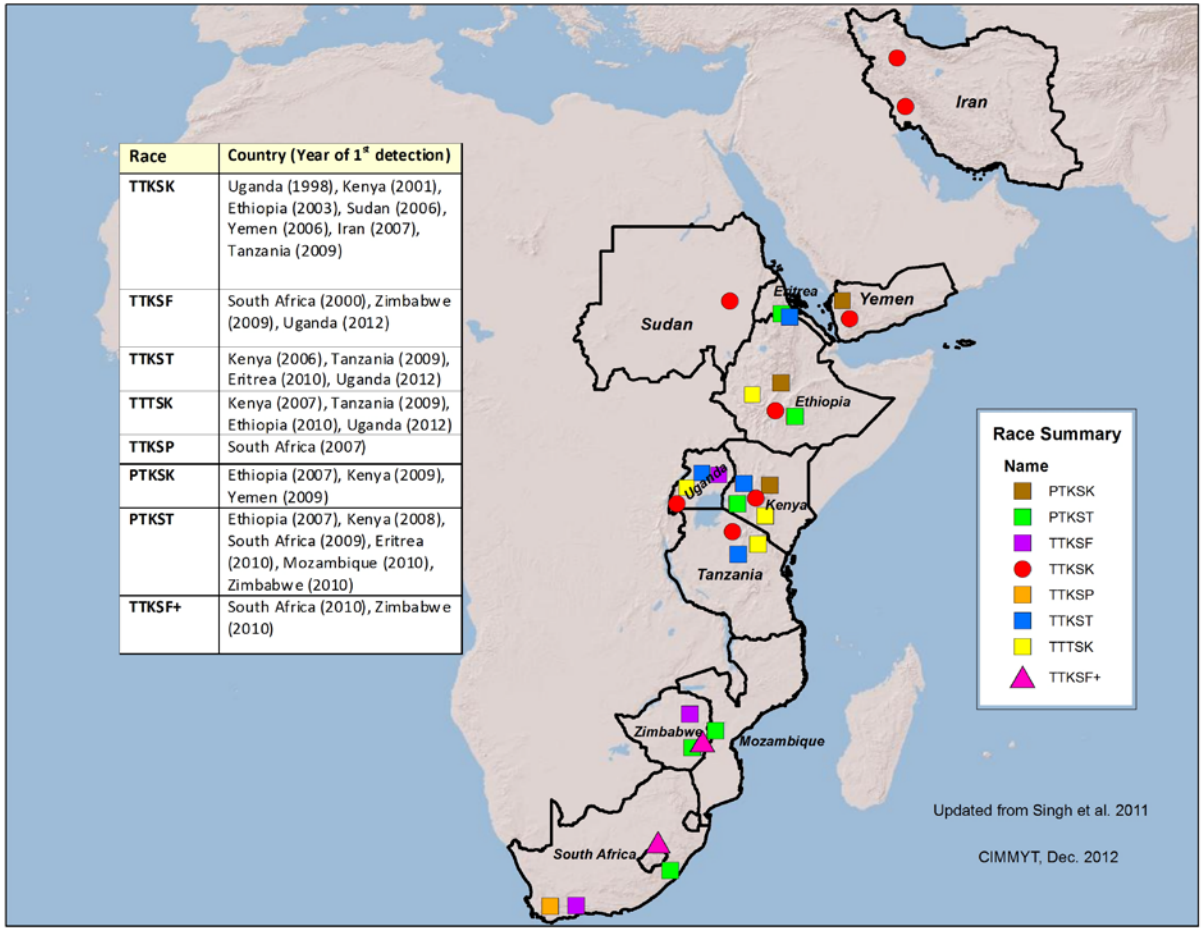
- First found in Uganda in 1998 and characterized in 1999.
- First race of *Pgt* with virulence to wheat stem rust resistance gene *Sr31*.
- Race designation: TTKSK
- Approximately 80% of the world wheat is susceptible to Ug99 and variants.

Ug99 Race Group (Ug99 RG)

Race	Key virulence difference				First found (Country and year)
	Sr31	Sr21	Sr24	Sr36	
TTKSK (Ug99)	Vir	Vir	Avir	Avir	Uganda, 1998
TTKSF/+	Avir	Vir	Avir	Avir	South Africa, 2000
TTKST	Vir	Vir	Vir	Avir	Kenya, 2006
TTTSK	Vir	Vir	Avir	Vir	Kenya, 2007
TTKSP	Avir	Vir	Vir	Avir	South Africa, 2007
PTKSK	Vir	Avir	Avir	Avir	Kenya, 2007
PTKST	Vir	Avir	Vir	Avir	Kenya, 2008

Based on Singh et al, 2011

Distribution of Ug99 Race Group



Race	Country (Year of 1st detection)
TTKSK	Uganda (1998), Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009)
TTKSF	South Africa (2000), Zimbabwe (2009), Uganda (2012)
TTKST	Kenya (2006), Tanzania (2009), Eritrea (2010), Uganda (2012)
TTTSK	Kenya (2007), Tanzania (2009), Ethiopia (2010), Uganda (2012)
TTKSP	South Africa (2007)
PTKSK	Ethiopia (2007), Kenya (2009), Yemen (2009)
PTKST	Ethiopia (2007), Kenya (2008), South Africa (2009), Eritrea (2010), Mozambique (2010), Zimbabwe (2010)
TTKSF+	South Africa (2010), Zimbabwe (2010)

RustTracker.org

Global response to Ug99

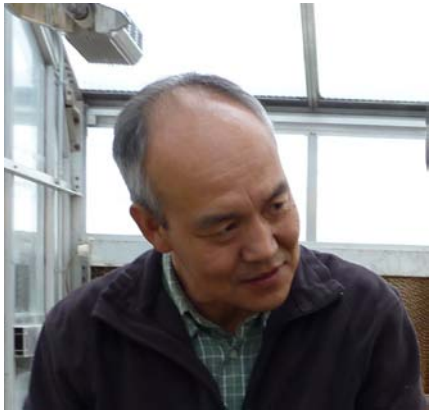
- Formation of the “Global Rust Initiative” in 2004, lead by Dr. Norman Borlaug. Later became the Borlaug Global Rust Initiative (BGRI).
- Developed a 10 point plan that included:
  - Monitor and characterize of the population *Pgt*.
  - Develop of trap plots and screening nurseries in Kenya and Ethiopia.
  - Screen existing wheat germplasm for resistance to Ug99.
  - Facilitate breading programs to develop resistance wheat varieties.

“Sounding the Alarm on Global Stem Rust”, May 2005

USDA ARS Cereal Disease Lab

USDA ARS Cereal Disease Lab

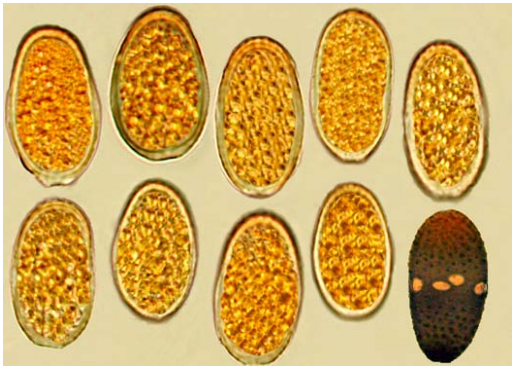
- CDL has played critical role in the global response to Ug99 and continues to be a leader.
- Only lab in the U.S. with the facilities and expertise to work with Ug99 and other critical isolates of *Pgt*.



Yue Jin

USDA ARS Cereal Disease Lab

- CDL has been working with foreign isolates of *Pgt* for over four decades.
- CDL uses multiple layers of containment.
  - Physical
  - Biological/Environmental



Containment: Physical

- CDL Building restricted access 24/7.
- Limited access (locked) greenhouses and growth chambers to personnel working on project.
- Use of protective clothing in work areas.
- All biological materials are sterilized after use.

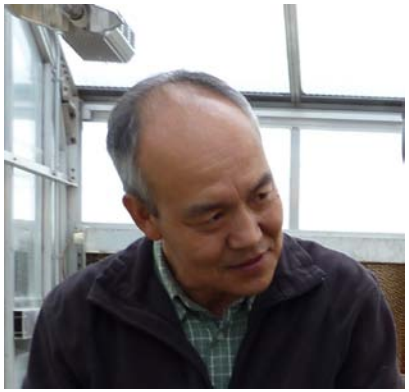
Containment: Biological/Environmental

- Work with Ug99 and other foreign isolates are restricted to the middle of the winter (Dec – Feb).
- Sever winter conditions in Minnesota provides a second layer of containment:
  - Eliminates any living host material required for growth and survival of *Pgt*.
  - If live spores of *Pgt* were to escape, spores would not survive.

Additional Facilities: St. Paul

- University of Minnesota & Minnesota Department of Agriculture – BL3 greenhouse and lab

CDL research Programs



- Yue Jin
  - Pathotyping of *Pgt* collections
    - Identified first two variants of Ug99
  - Screening of wheat and wild relatives for resistance to Ug99 RG.
  - Characterizing barberry (sexual host of *Pgt*) populations in Africa and Central Asia.
    - Determined that barberry species common in Kenya and Ethiopia is susceptible to *Pgt*.

CDL research Programs

- Matt Rouse
  - Identification and characterization of stem rust resistance genes.
    - Developed high-through put screening system.
      - Phenotyped 40,000 lines in the winter of 2011-2012
  - Genetic mapping and cloning of *Sr* genes.
    - *Sr*28, *SrGabo56*, *SrTmp*, *SrMVZelma*
  - Development of “Ug99” resistant germplasm for breeding programs.

CDL research Programs

- Les J. Szabo
  - Genomics of *Pgt*.
    - Characterization of *Pgt* genome.
    - Identification and characterization of effector genes.
  - Genetic characterization of global populations of *Pgt*.
  - Development of rapid molecular diagnostic systems for *Pgt*.
    - Developed two stage assay system for rapid identification of Ug99 RG and prediction of race phenotype based on SNP genotype.

Challenges

Challenges

- Capacity
  - Currently, only two laboratories in world are able to work with Ug99 RG and have the technical skill.
    - A new addition is in the process of being built at the USDA ARS CDL, but this will not meet the demand.
  - Shortage of properly trained scientists worldwide to work on *Pgt* the other cereal rust pathogens.
    - International organizations (BGRI, CIMMYT, ICARDA, PBI) are working on training the next generation.

Challenges

- Biological materials
  - Lack of access to regions/countries to make surveys and collections *Pgt*.
  - Restrictions on sharing (collections, DNA and data) and movement of biological materials.
  - Appropriate transport of living *Pgt* collections.
    - Often less than 10% of the cultures survey shipment to the CDL from Africa.

Take home message

- Current international efforts have made remarkable progress in combating “Ug99” in less than 10 years.
- International efforts needs to be increased and sustained in order to successfully control “Ug99”.
- Cereal rust fungi are “shifty enemies” and the current epidemics of wheat stem rust and wheat stripe rust will not be the last.

Acknowledgements

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Extras

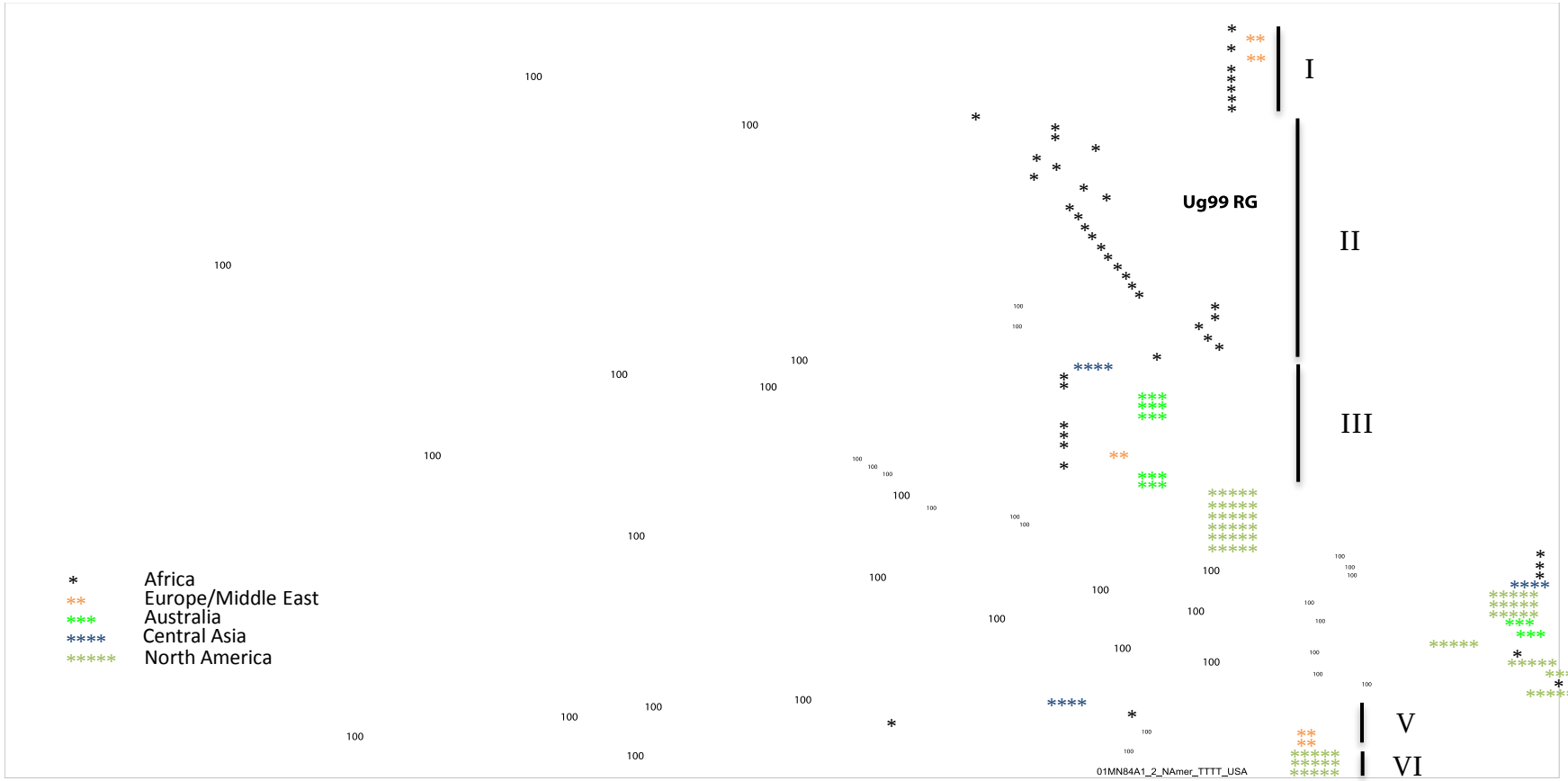
Molecular diagnostics – Ug99 RG

- Two stage Assay
  - Stage-1
    - Is it Ug99 RG?
  - Stage -2
    - What member of the Ug99 RG?
- Samples
  - Stem segment with a single pustule is collected.
  - Sample is ethanol killed.
  - Sent to central lab for analysis.

Sample set from Kenya (2012)

- 43 samples (D12KEN101-143)
- Stage-1 assay
  - All samples were positive for Ug99 RG.
- Stage-2 assay
  - TTKSK (23 samples) – 2 different genotypes
  - TTKST (17 samples)
  - TTTSK (1 sample)
  - Two samples were inconclusive.
    - Likely mixed samples.

Phylogenetic analysis of *Pgt*



Maximum parsimony, 5,000 bootstrap replicates

Cuomo, Szabo & Sakthikumar, unpublished data