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Characterization and molecular identification of *Phytophthora* spp. associated with Phytophthora blight of vegetables in Texas.

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PLANT PATHOLOGY & MICROBIOLOGY

AgriLIFE EXTENSION
Texas A&M System

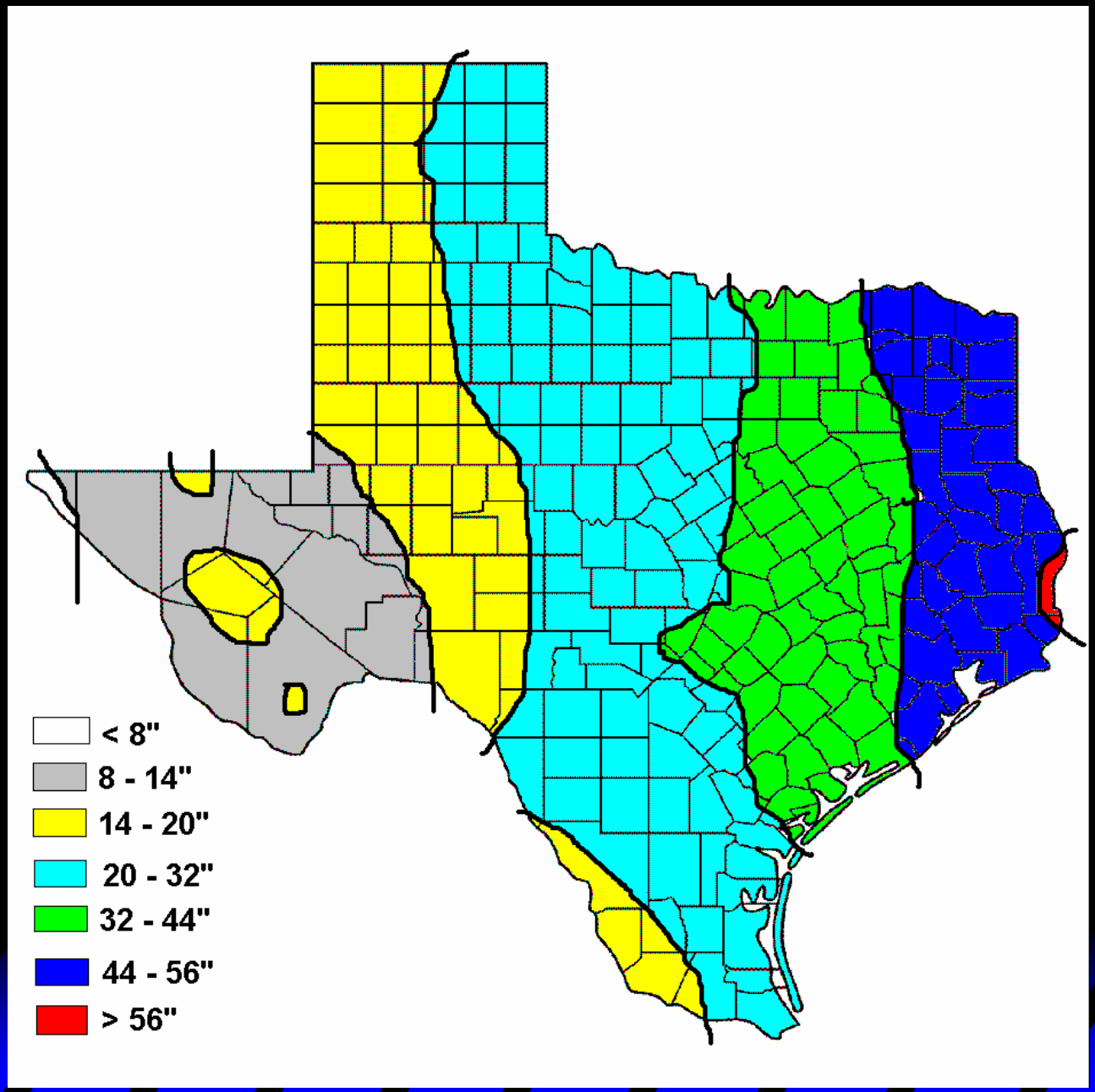
Texas High Plains Plant Diagnostic Laboratory

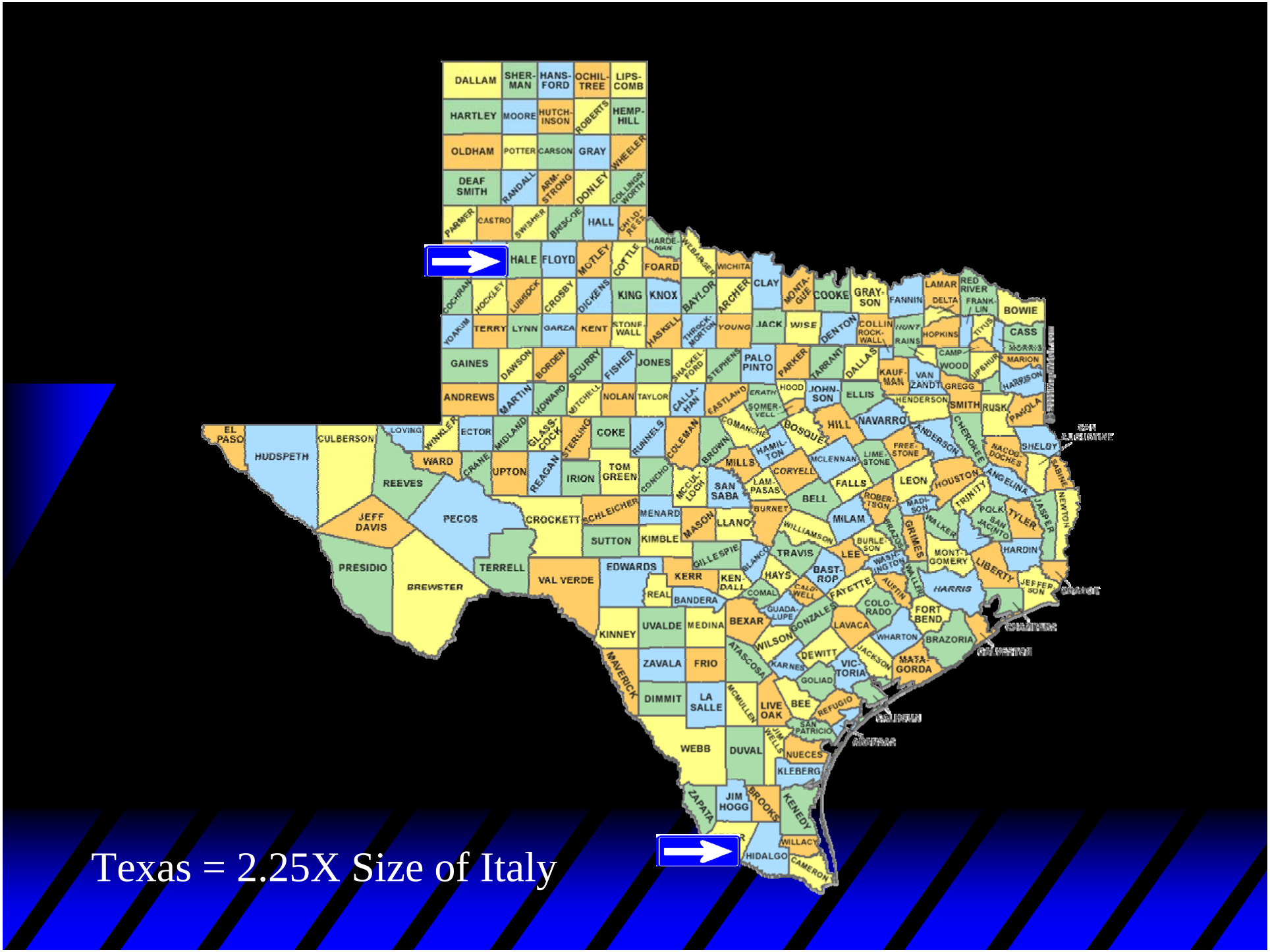
Texas AgriLife Research and Extension Center
6500 Amarillo Blvd. W
Amarillo, Texas 79106

PLANT PATHOLOGY & MICROBIOLOGY

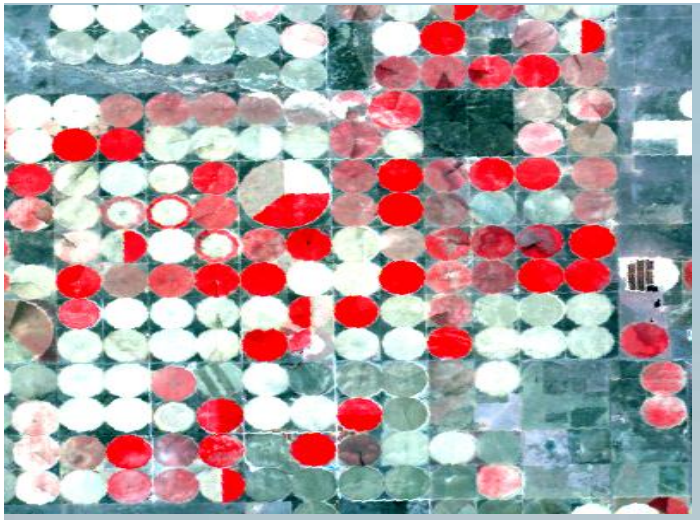
TEXAS TECH UNIVERSITY
est. 1923

TEXAS RAINFALL: GO EAST, GET WET



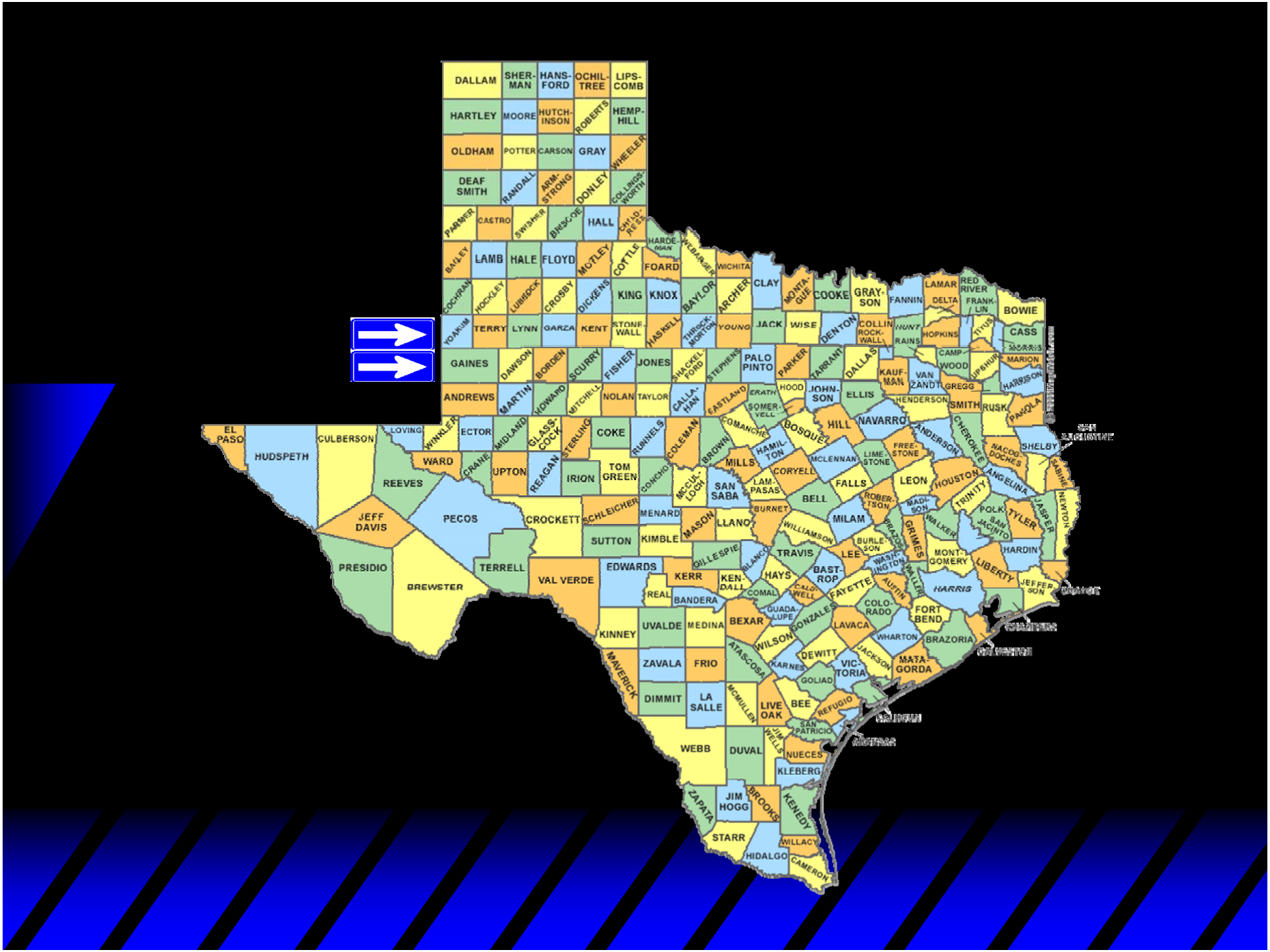


Texas = 2.25X Size of Italy



Phytophthora blight

- Caused by *Phytophthora capsici* (oomycete)
- Devastating on pepper, cucurbits, tomato, and eggplant
- Spread by surface water, wind and splashing rains.
- Present in Texas for over 60 years.


DALLAM
SHERMAN
HANSFORD
OCHILTREE
LIPSCOMB
HARTLEY
MOORE
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ROBERTS
HEMPHILL
OLDHAM
POTTER
CARSON
GRAY
WHEELER
DEAF SMITH
RANDALL
ARMSTRONG
DONLEY
COLLINGSWORTH
PARMER
CASTRO
SWISHER
BRISCOE
HALL
CHILDRESS
BAILEY
LAMB
HALE
FLOYD
MOTLEY
COTTLE
FOARD
HARDEMAN
WILBARGER
WICHITA
CLAY
MONTAGUE
COOKE
GRAYSON
FANNIN
LAMAR
RED RIVER
BOWIE
COCHRAN
HOCKLEY
LUBBOCK
CROSBY
DICKENS
KING
KNOX
BAYLOR
ARCHER
YOAKUM
TERRY
LYNN
GARZA
KENT
STONEWALL
HASKELL
THROCKMORTON
YOUNG
JACK
WISE
DENTON
COLLIN
ROCKWALL
HUNT
RAINS
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TITUS
CASS
MORRIS
GAINES
DAWSON
BORDEN
SCURRY
FISHER
JONES
SHACKELFORD
STEPHENS
PALO PINTO
PARKER
TARRANT
DALLAS
KAUFMAN
VAN ZANDT
CAMP
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HOWARD
MITCHELL
NOLAN
TAYLOR
CALLAHAN
EASTLAND
ERATH
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HENDERSON
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PANOLA
EL PASO
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CULBERSON
LOVING
WINKLER
ECTOR
MIDLAND
GLASSCOCK
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RUNNELS
COLEMAN
BROWN
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ANDERSON
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NACOGDOCHES
SHELBY
SABINE
SAN AUGUSTINE
WARD
CRANE
UPTON
REAGAN
IRION
TOM GREEN
CONCHO
MILLS
HAMILTON
MCLENNAN
LIMESTONE
FREESTONE
ANGELINA
REEVES
MCCULLOCH
SAN SABA
CORYELL
FALLS
LEON
HOUSTON
TRINITY
JEFF DAVIS
PECOS
CROCKETT
SCHLEICHER
MENARD
MASON
LLANO
BURNET
LAMPASAS
BELL
ROBERTSON
MADISON
POLK
TYLER
JASPER
NEWTON
SUTTON
KIMBLE
WILLIAMSON
MILAM
BRAZOS
GRIMES
WALKER
SAN JACINTO
PRESIDIO
BREWSTER
TERRELL
VAL VERDE
EDWARDS
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GUADALUPE
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COLORADO
FORT BEND
KINNEY
UVALDE
MEDINA
BEXAR
LAVACA
WHARTON
BRAZORIA
WILSON
ATASCOSA
DEWITT
JACKSON
MATAGORDA
MAVERICK
ZAVALA
FRIO
KARNES
GOLIAD
VICTORIA
DIMMIT
LA SALLE
MCMULLEN
LIVE OAK
BEE
REFUGIO
SAN PATRICIO
WEBB
DUVAL
JIM WELLS
NUECES
KLEBERG
ZAPATA
JIM HOGG
BROOKS
KENEDY
STARR
WILLACY
HIDALGO
CAMERON

Symptoms

P. capsici in Texas High Plains

- 2006: First known report on pumpkin and winter squash (Isakeit, 2007): 36 acres
  - 2% field affected August; 78% lost next month
  - Yoakum County
- 2007:10-12 acres of chile pepper in a 60 acre field affected by this pathogen.
  - Gaines County

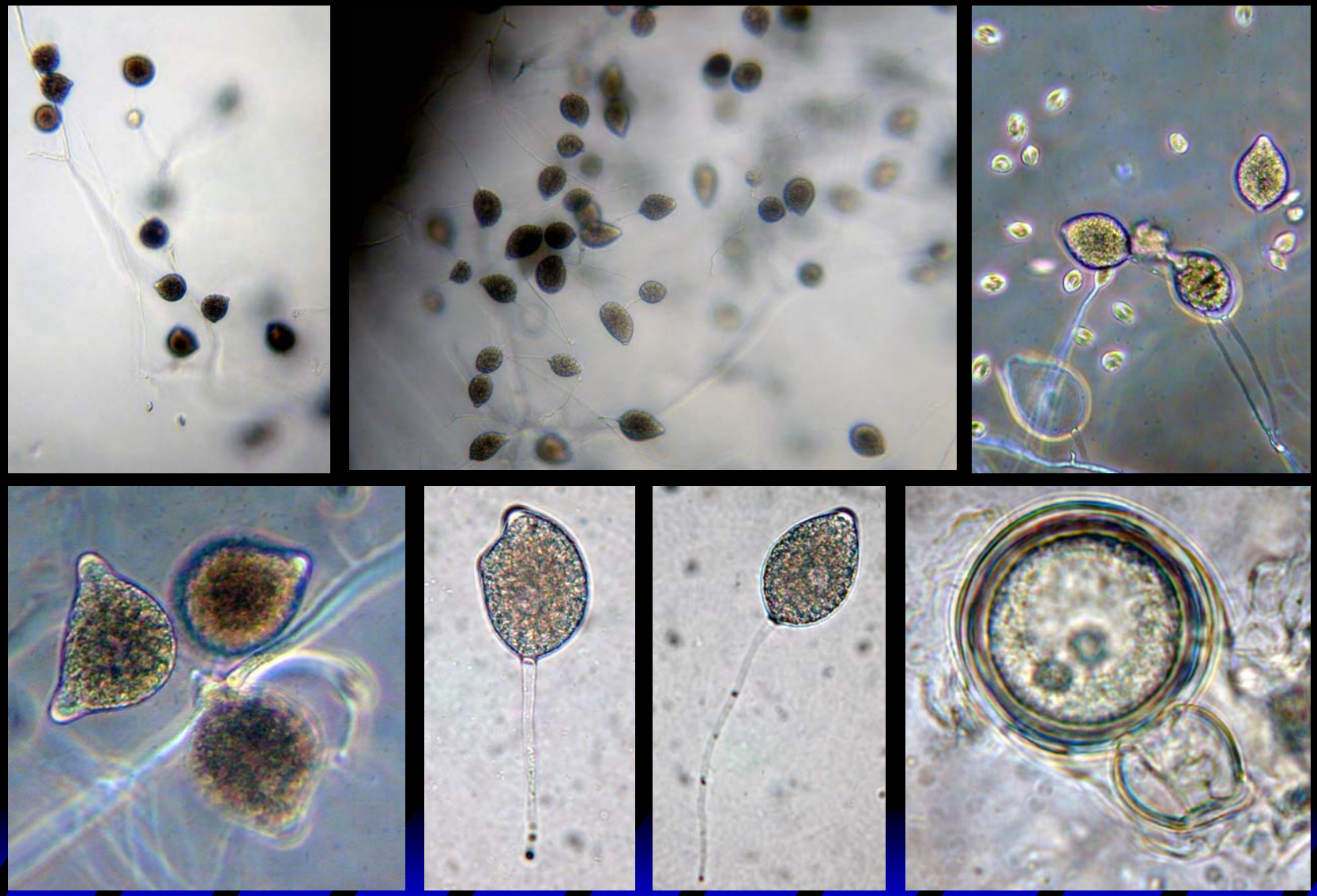
Morphological

- These isolates had semi-papillate to papillate sporangia, caducous sporangia with pedicels that exceeded 35 µm in length.
- Sporangia 37 to 40 X 21 to 23 µm
- Exhibited heterothallism; no chlamydospores.
- When paired with an opposite mating type, oospores produced were mostly aplerotic.
- Oogonia were amphigynous in their antheridial attachment.
- Oospores 25 to 27 µm in diameter.

French-Monar et al. 2006. Plant Dis. 90: 345-350

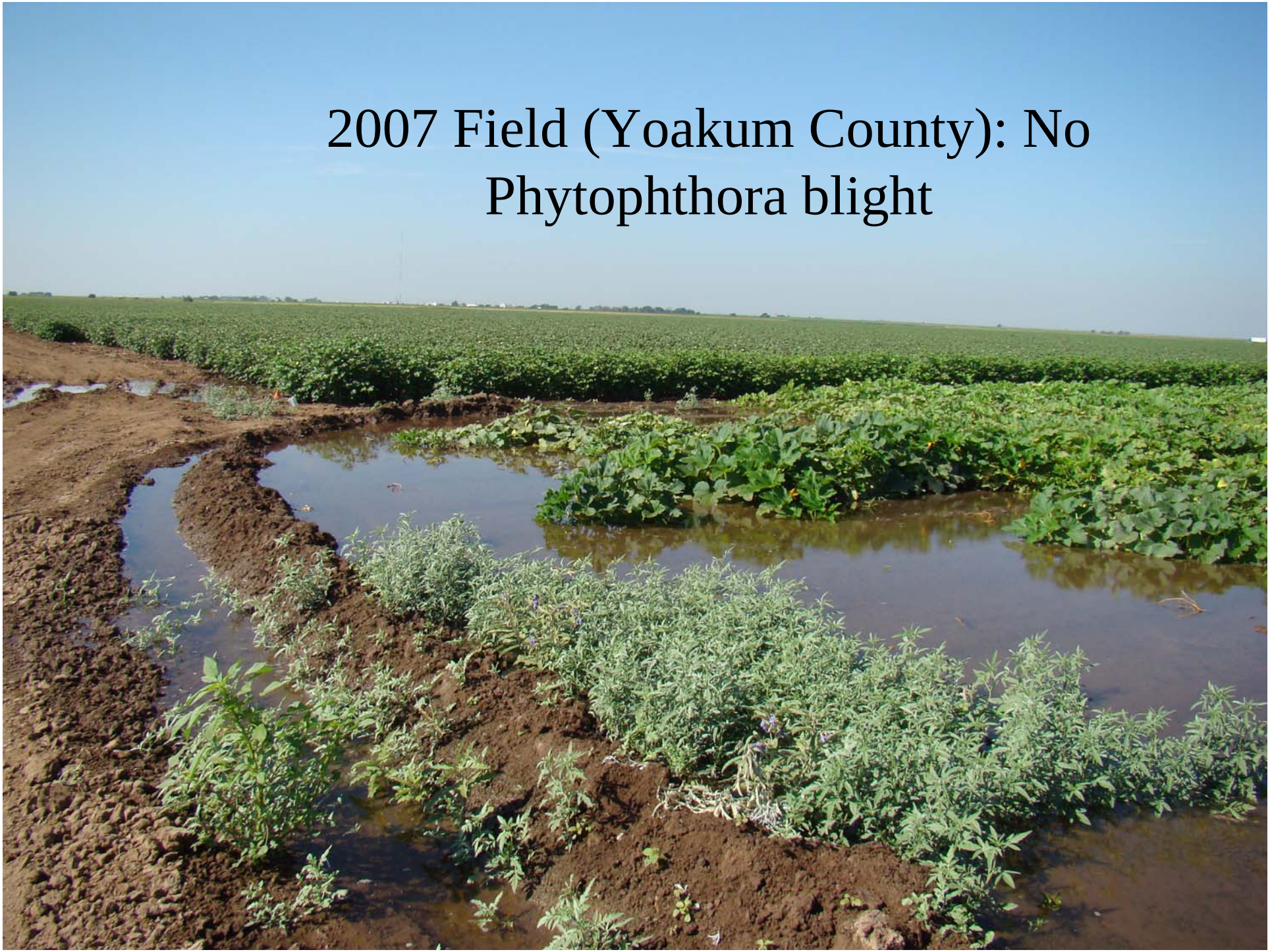
Isakeit, T. 2007. Plant Dis. 91: 633

Phytophthora capsici from *Capsicum annuum*



Photos by Gloria Abad, Ph.D USDA/APHIS/MDL 8.18.08

2007 Field (Yoakum County): No Phytophthora blight



2007 Field (Gaines County)

Phytophthora blight on peppers

Ronald French

(Texas A&M-TCE)

Phytophthora blight on bell pepper

Phytophthora blight in Texas

Chile pepper

Phytophthora sp. on watermelon

Texas

Compatibility type determination

- For compatibility type determination:
  - a 6-mm mycelial plug of a known A1 or A2 mating type is added to one side of a clarified V8 juice agar plate.
  - on the opposite side, the isolate of unknown mating type is added to the agar plate.
  - After 7 days, the center of the plate is observed under the microscope for the oospore production.

Isolates of Phytophthora

Isolate	Compatibility Type
Ph 204 (Chile pepper-S. Texas)	A1
Ph 205 (Winter squash-N. Texas)	A2
Ph 206 (Pumpkin-N. Texas)	A2
Ph 207 (Chile pepper-Mexico)	--
Ph 211 (Chile pepper-N. Texas)	A1
Ph 212 (Chile Pepper-N. Texas)	A1
Ph 213 (Chile pepper-N. Texas)	A2
Ph 214 (Chile pepper-N. Texas)	A2
Ph 216 (Watermelon-S. Texas)	A1

Methodology (cont.)

- For mefenoxam sensitivity studies:
  - 10% clarified V8 juice agar was amended with 0, 5, and 100 µg/ml of mefenoxam (Ridomil Gold EC).
  - A plug of mycelium is set at the center of each plate.
  - After three days, colony diameters are measured at each concentration.
  - Growth of >40% of control at 100 µg/ml= Resistant
  - Growth of >40% of control at 5 µg/ml = Intermediate
  - Growth of <40% of control= Sensitive.

Sensitivity to mefenoxam

Isolate (compatibility type)		Percent growth	
		5 ppm	100 ppm
Ph 204 (Chile pepper-S. Texas)	A1	22	26
Ph 205 (Winter squash-N. Texas)	A2	33	27
Ph 206 (Pumpkin-N. Texas)	A2	30	24
Ph 207 (Chile pepper-Mexico)	--	**42**	**48**
Ph 211 (Chile pepper-N. Texas)	A1	15	24
Ph 212 (Chile Pepper-N. Texas)	A1	18	24
Ph 213 (Chile pepper-N. Texas)	*A2*	19	25
Ph 214 (Chile pepper-N. Texas)	A2	16	13
Ph 216 (Watermelon-S. Texas)	A2	21	31

Sensitive fungal isolate

0 ppm

5 ppm

100 ppm

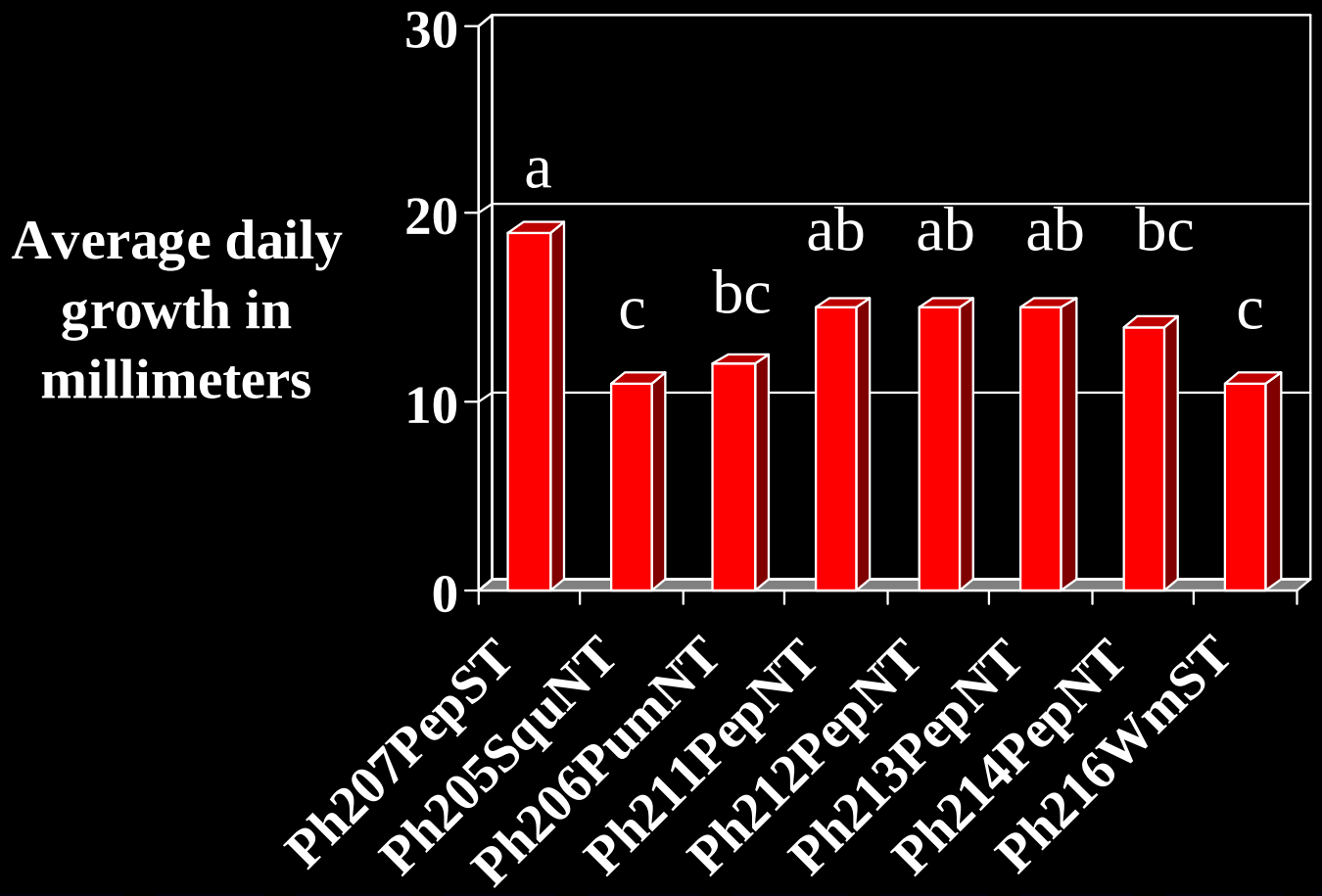
Fully resistant isolate

0 ppm

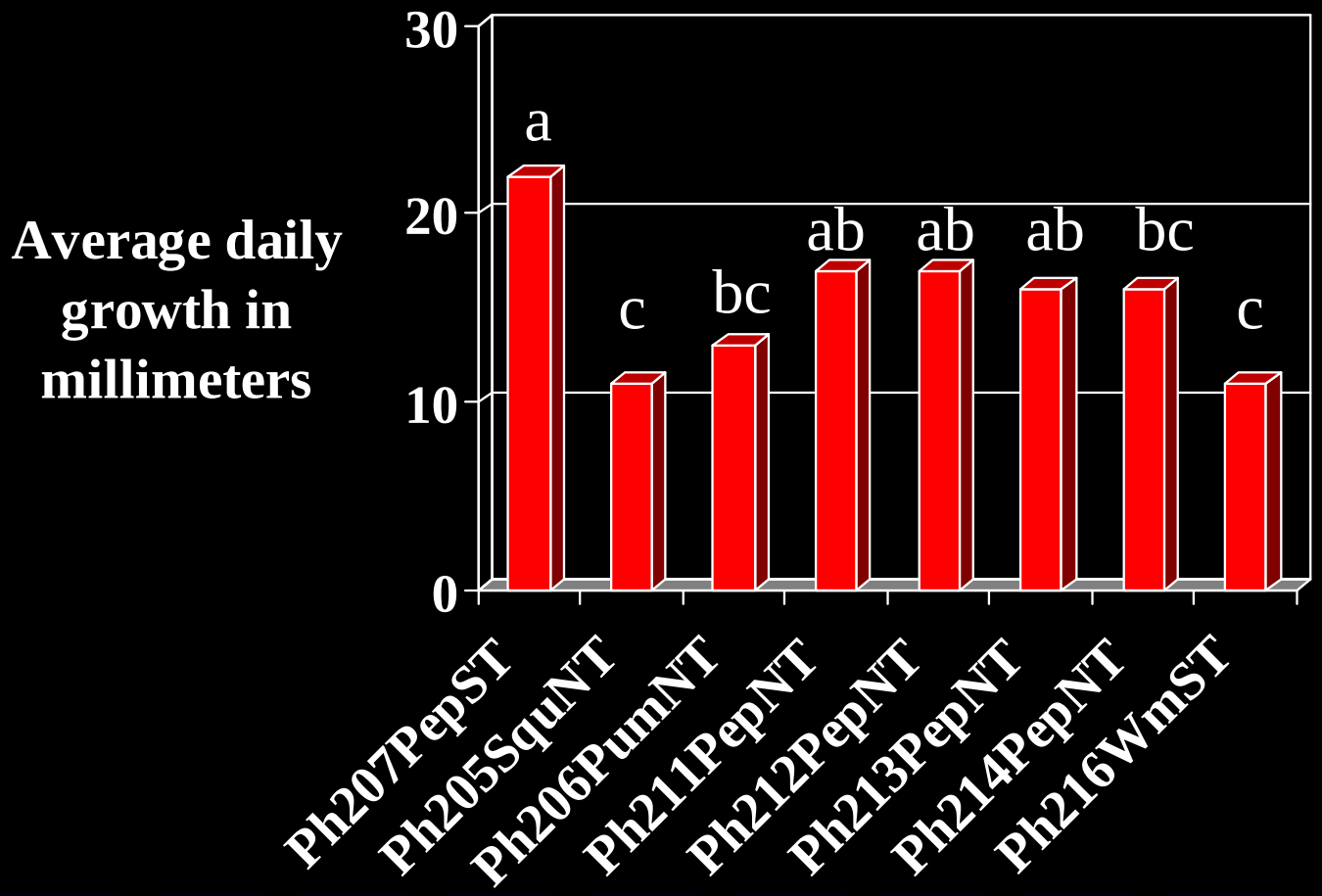
5 ppm

100 ppm

Growth rate at 25° C



Growth rate at 30° C

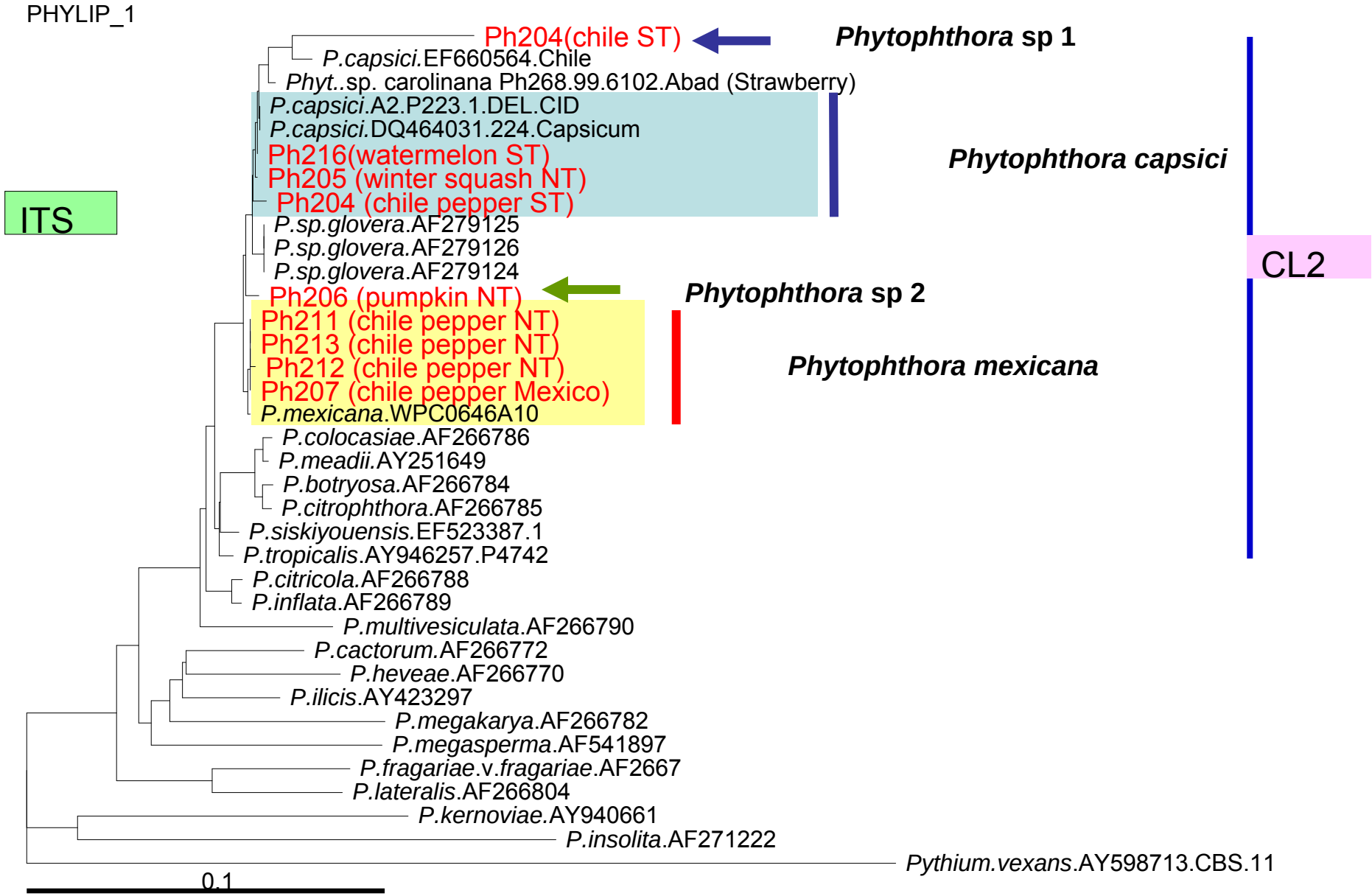


ITS Phylogeny

- Isolates from diverse backgrounds were grown in clarified V8 juice broth.
- Isolates were lyophilized.
- DNA extraction using Qiagen DNeasy Kit.
- PCR amplification
  - ITS1, 5.8 S, ITS 2 region (Nuclear)= 752 bp
    - Primers ITS 4 and ITS 5
    - Temperature cycle parameters: 93°, 53°, 72°

ITS Phylogeny

- Neighbor joining phylogenetic tree with 1000 bp based on the ITS1-5.8S-ITS2 complete region of *Phytophthora* Clade 2 (Cooke et al 2000).
- Tree shows the position of samples from vegetables in Texas to representative sequences of *P. capsici* and other known taxa in the clade.
- *Pythium vexans* is outgroup.
- Scale bar unit: number of nucleotide substitutions per site.



Neighbor joining phylogenetic tree with 1000 bs based on the ITS1-5.8S-ITS2 complete region of *Phytophthora* Clade 2 (Cooke et al 2000) showing the position of samples from vegetables in Texas to representative sequences of *P. capsici* and other known taxa in the clade. *Pythium vexans* is outgroup. Scale bar unit: number of nucleotide substitutions per site.

Phy. sp. caroliniana Ph268.99.6102 from strawberry NC- Homothallica

- How can the pathogen survive and persist in the Texas High Plains?

Common Purslane (*Portulaca oleracea*)

Andy Bennett

French-Monar et al. 2006. *Plant Dis. 90:345-350.*

Carolina Wild Geranium (*Geranium carolinianum*)

Daniela Bell

French-Monar et al. 2006. *Plant Dis. 90*:345-350.

Nightshades (*Solanum spp.)*

http://www.agf.gov.bc.ca

French-Monar et al. 2006. *Plant Dis. 90:345-350.*

Conclusions

- Phytophthora blight devastation in Texas may not be solely the responsibility of *P. capsici*
- Up to 4 species of Phytophthora might be involved in Phytophthora blight of vegetables.
- Isolates from watermelon (2008, S. Texas), winter squash (2006, N. Texas) and chile pepper (collection, S. Texas) had a perfect alignment for *P. capsici.*

Conclusions

- *Isolates from chile pepper in N. Texas perfectly aligned with P. mexicana.*
- The pumpkin isolate (N. Texas) did not group with any Phytophthora sp. and could represent a new species.
- Chile pepper isolate from S. Texas did not grouped with another isolate from chile pepper (Mexico) and closely related to a species to be reported by G. Abad on strawberry.

Thank You