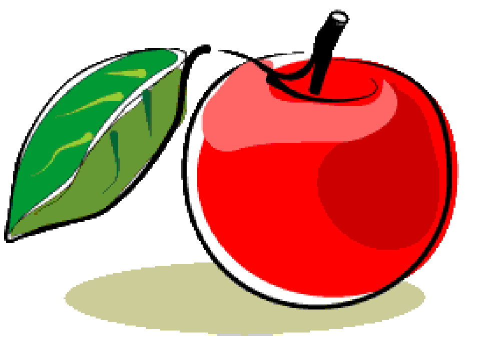
Fire Blight: Streptomycin Resistance Updates and Management Strategies

Kerik D. Cox & Kiersten Bekoscke
NYSAES

Plant Pathology and Plant-Microbe Biology Section
School of Integrative Plant Science
Cornell University



Outline

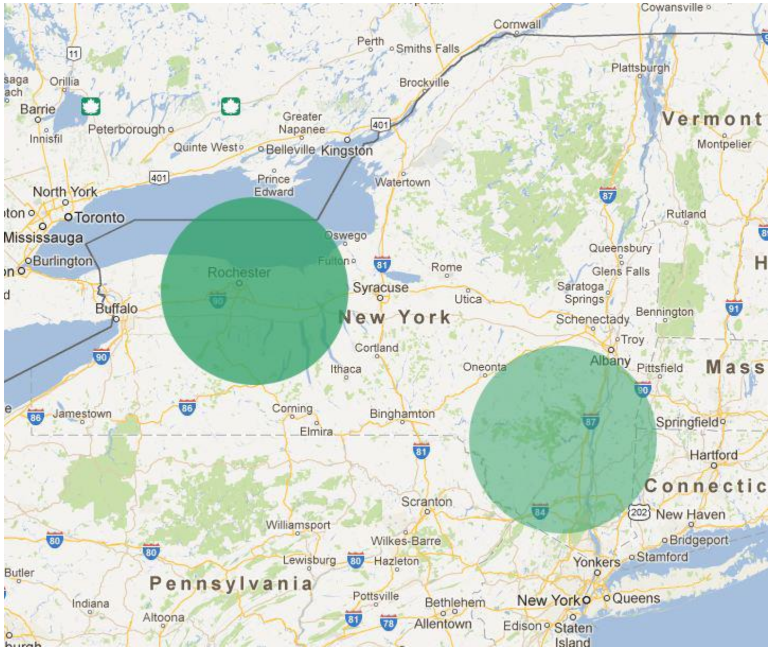
- Streptomycin resistance update
  - History and status
  - Selection & origin
- Managing fire blight
  - Pre & post-season
  - Bloom
  - Post-bloom & Summer

History of streptomycin resistance in NY

- 2002
  - SmR Ea in two neighboring orchards in Wayne County: Trees from a nursery with SmR Ea
  - Growers removed the orchards
- 2003-2011
  - Occasional testing of fire blight samples: 2011 detection of SmR Ea at 4 new locations
- 2012-2014
  - Formal survey: partnered LOFT, CCE ENYCHP, and NYS IPM

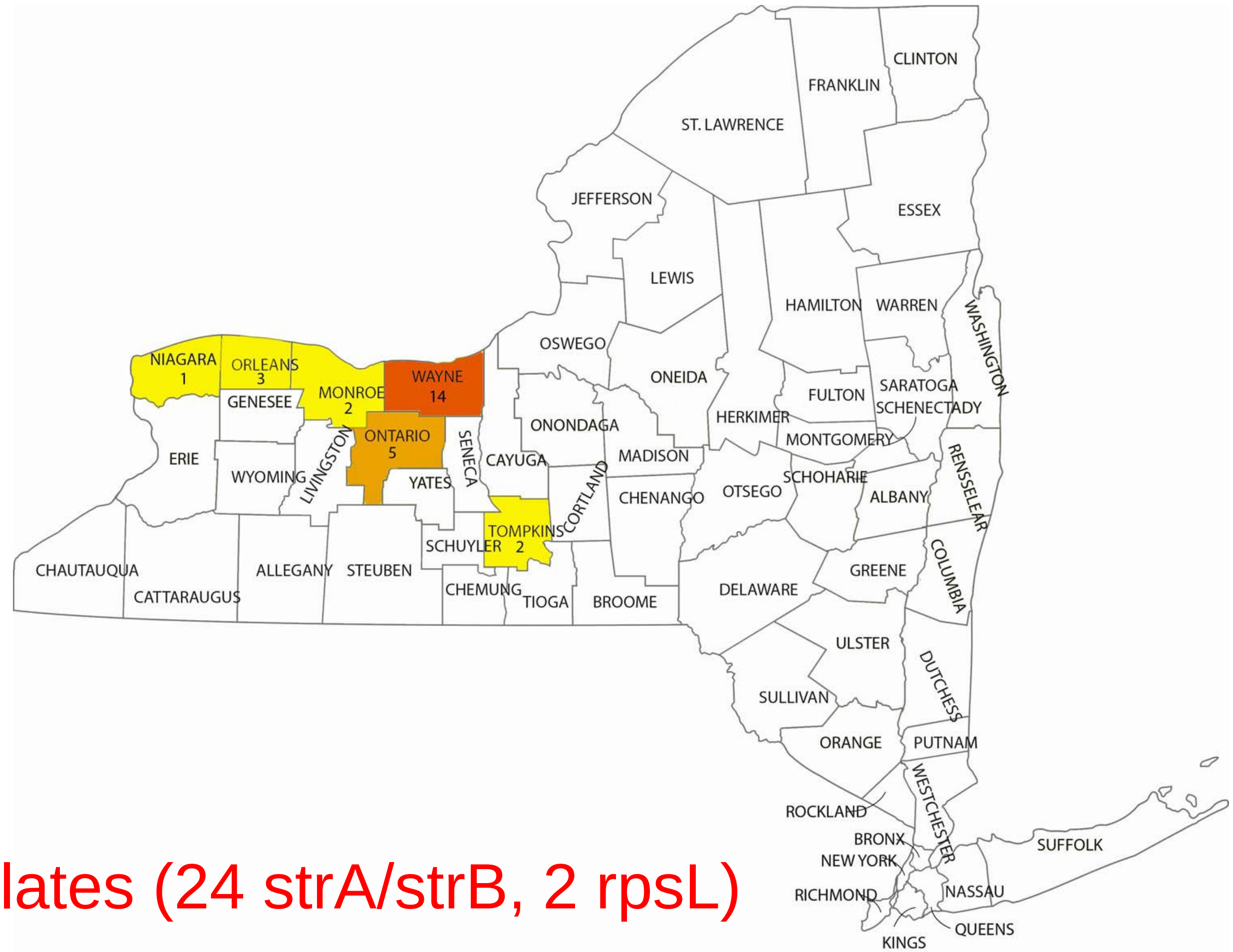
Status of streptomycin resistance

- 2012: 175 isolates from 43 commercial sites
- 2013: 320 isolates from 32 commercial sites
- 2014: 800 isolates from 32 commercial sites
  - Majority of sampling: Lake Ontario & Hudson Valley



Status of streptomycin resistance

- 2012-2013: 16 apple production operations had SmR Ea



26 Sm^R Isolates (24 strA/strB, 2 rpsL)

Trends & implications

- 2014: Lots of Ea, but no SmR Ea
  - Other tree declines present (1/3 samples > BSB, winter injury, & fungal decay blights)
- SmR Ea seems to be restricted to western NY
  - Closest to regions of previous outbreaks
  - Eastern NY appears to be SmR Ea free
- 16 apple production operations had SmR Ea
  - Disproportionate ratio of SmS to SmR strains

Trends & implications

- All samples from shoot blight: no catastrophic strep failures w/ widespread losses
  - Numerous reports of FB in RubyFrost, Honeycrisp, & Gala

- In “high risk” areas for strep resistance
  - Streptomycin and oxytet mixes: Seemed to improve overall control of FB in Western NY
  - High risk operations with SmR Ea in 2012 had only SmS in 2013 & 2014

Outline

- Streptomycin resistance update
  - History and status
  - Selection & origin
- Managing fire blight
  - Pre & post-season
  - Bloom
  - Post-bloom & Summer

Selection and Origin

- Effect of varying streptomycin and kasugamycin use on the selection of streptomycin resistant isolates of *E. amylovora* and other epiphytes

- Use CRISPR strain tracking to better understand the origin of streptomycin resistant strains of *E. amylovora*

Selection of strep resistance

- Dogma: applying strep after bloom for shoot blight > strep resistance
- *Erwinia amylovora* is not a good leaf/shoot epiphyte – Rif+ strains
- The evidence is anecdotal
  - Transfer of strep resistant from epiphytes not proven in the lab or field, yet!
  - More strep sprays = more Sm^R bacteria

Selection of strep resistance

- Loper 1991: Strep use recovery of Sm^R *E. amylovora*
  - Orchards with 2-10 years with 1-5 applications / year correlates with recovery of Sm^R *E. amylovora*

- Yashiro and McManus 2012: Streptomycin use & phyllosphere bacteria
  - No treatments or enumeration of species
  - No correlation > streptomycin use & recovery of Sm^R bacteria
  - High populations of bacteria with intrinsic and stable streptomycin resistance (*Pseudomonas)*

Selection of strep resistance

- How do increasing applications of Agri-Mycin 17 or Kasumin 2L effect epiphytic bacteria in the apple phyllosphere?

- Increasing streptomycin applications > increased Sm^R epiphytes

- Increasing kasugamycin applications: No effect on abundance of Sm^R epiphytes

Selection of strep resistance

10 yr. old ‘Idared’: Agrimycin 24 oz/A or Kasumin 2L 64 fl oz/A

- **Control**
- **3 applications**
- **5 applications**
- **10 applications**

50 leaves collected per tree
(4 replications)

Collections

1: 5/20 – 6/3 (Before applications)
2: 6/10 – 6/24 (After 3 applications)
3: 6/24 – 7/8 (After 5 applications)
4: 7/29 – 8/14 (After 10 applications)

Selection of strep resistance

Leaf Collection

Sonication

Dilution Plating

Crosse Goodman Media with and without streptomycin and kasugamycin

Identification

Morphology

PCR

Pseudomonas sp.: 16S rRNA

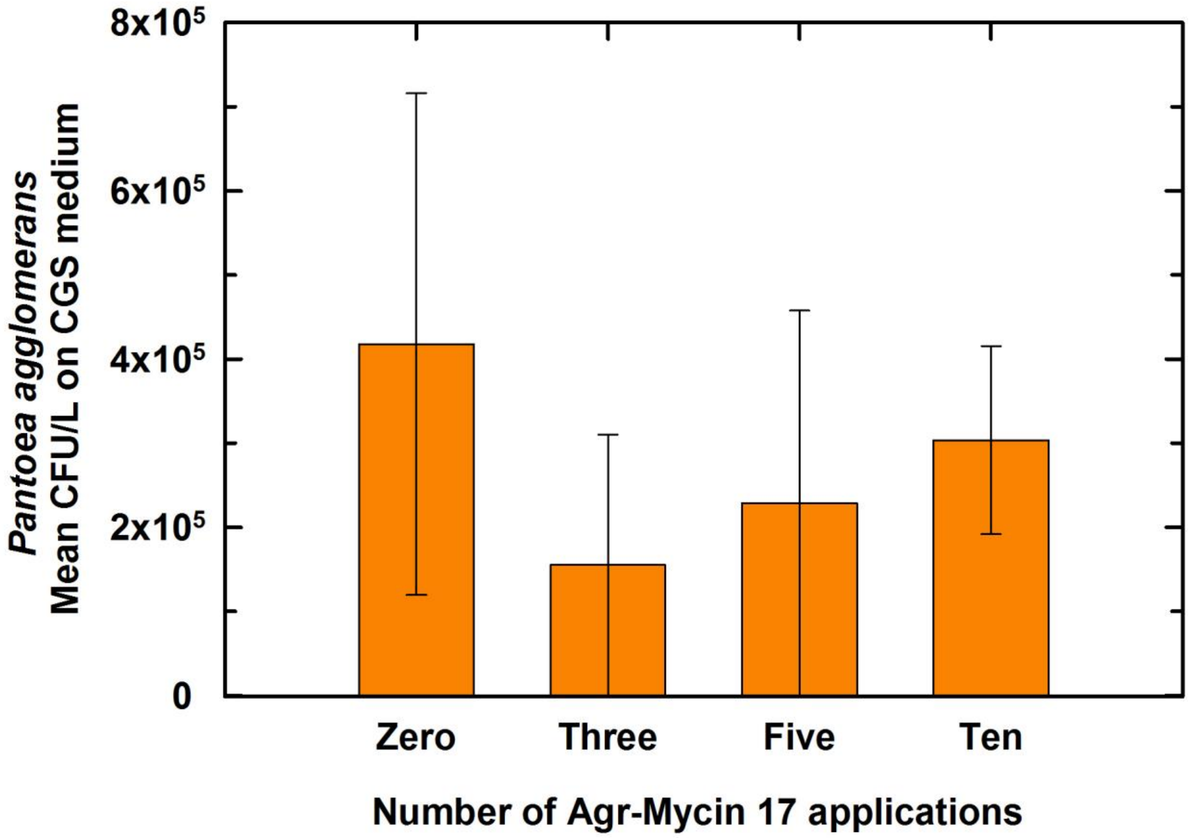
P. agglomerans: *pag2R*

E. amylovora: pEa29

Strep resistance genes: strA/B

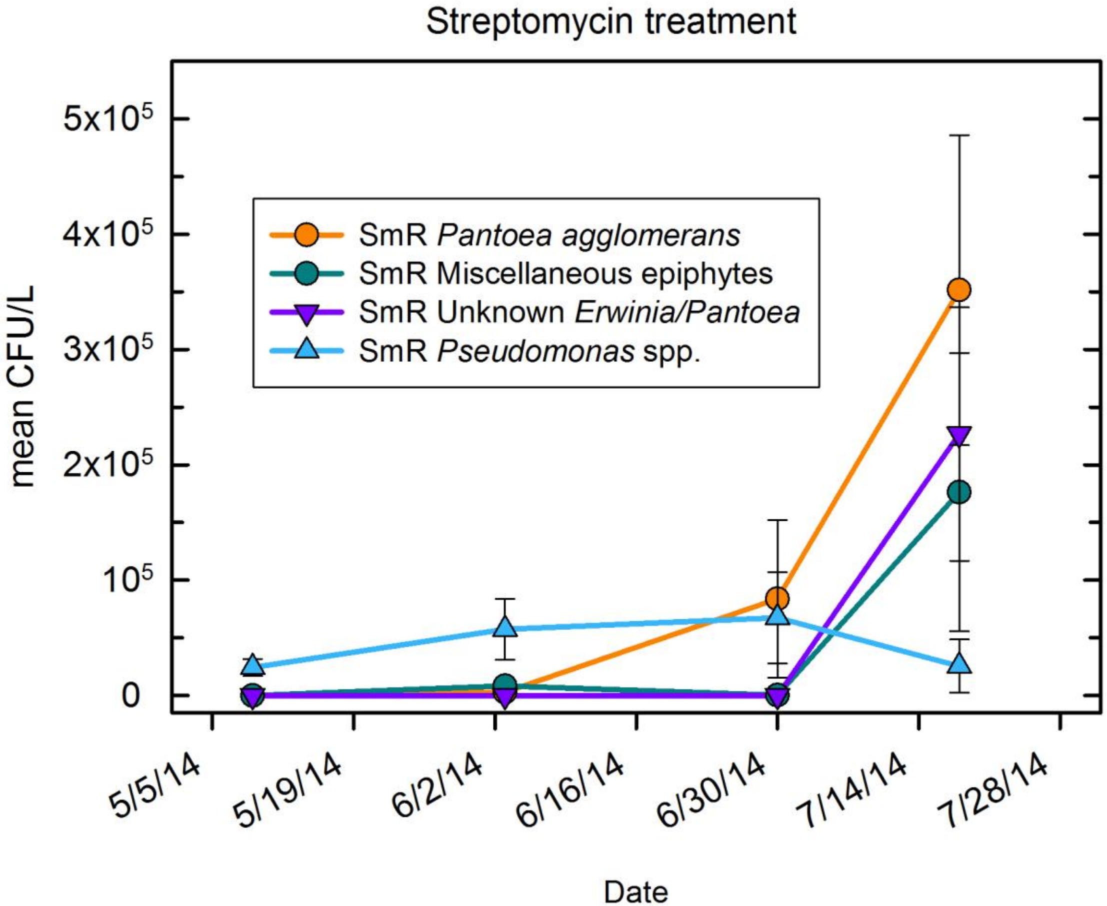
Streptomycin application results

- Five different epiphytic bacteria groups
  - No significant differences for any epiphytes after 0, 3, 5, and 10 applications



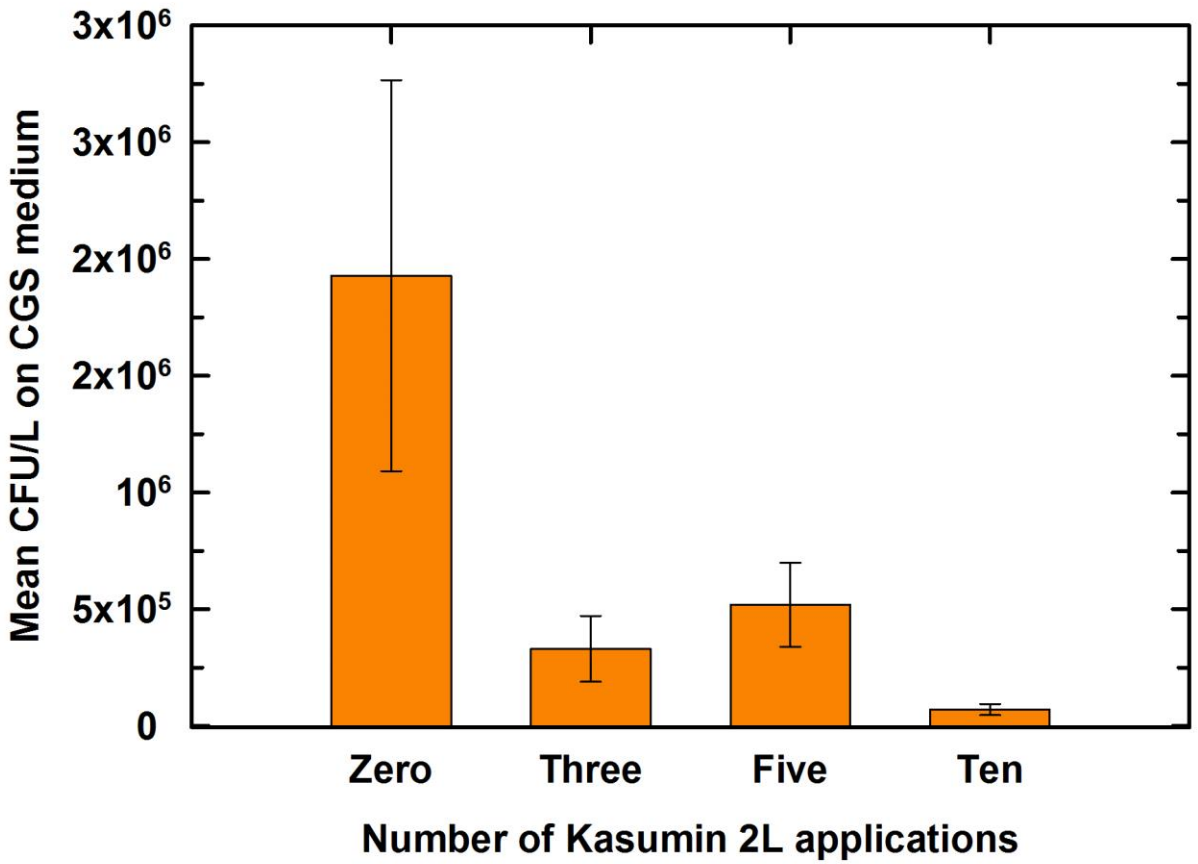
Streptomycin application results

- Changes in epiphytes over time
  - Epiphytic *E. amylovora* nearly absent
  - Higher pops. Sm^R epiphytes at end of season



Kasugamycin application results

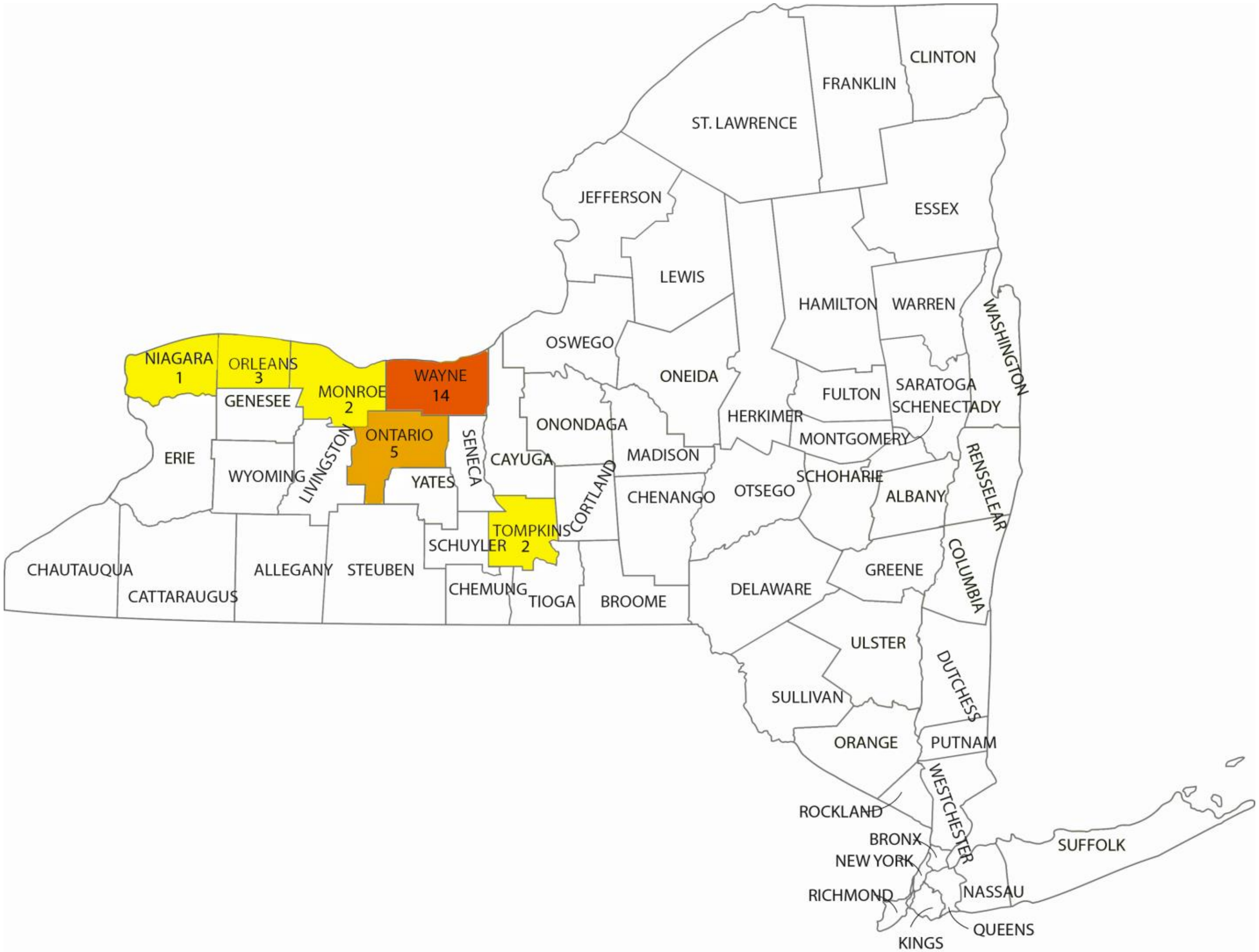
- All Sm^R epiphytes decrease with increasing Kasumin 2L applications



Selection of resistance summary

- Up to 10 applications of streptomycin did not effect abundance Sm^R *E. amylovora* in field studies
- Increasing applications of streptomycin may increase total Sm^R bacteria
- Increasing kasugamycin applications may decrease total Sm^R bacteria

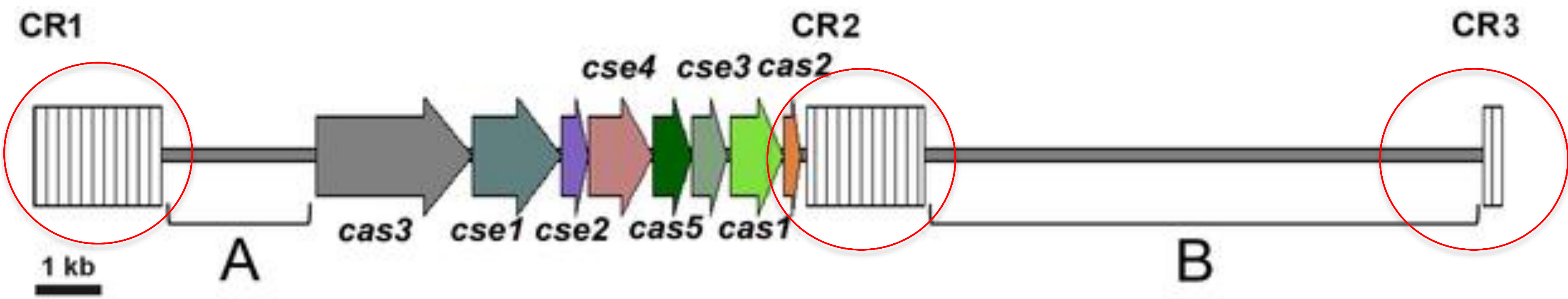
Origin of strep resistance



26 Sm^R Isolates (24 strA/strB, 2 rpsL)

Origin of strep resistance

Clustered **R**egularly **I**nterspaced **P**alindromic **R**epeats



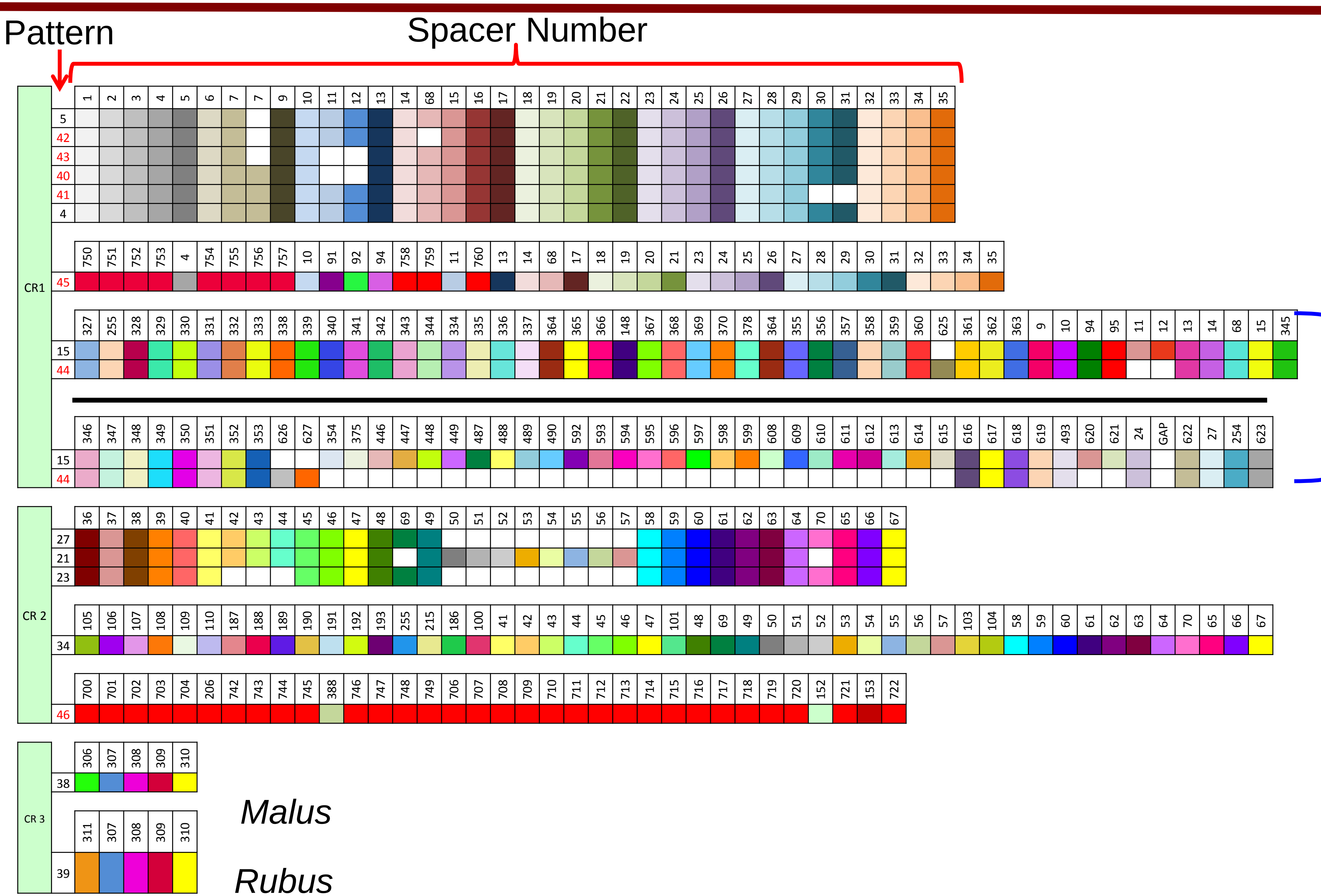
McGhee GC, Sundin GW (2012) Erwinia amylovora CRISPR Elements Provide New Tools for Evaluating Strain Diversity and for Microbial Source Tracking. PLoS ONE 7(7): e41706. doi:10.1371/journal.pone.0041706
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0041706

- Found in ~48% of bacteria
- Adaptive immune system: recognition of foreign DNA
- Spacer arrays:
  - Temporal organization and polarity
  - Used to investigate divergence and phylogenetic relationships (*E. amylovora*, *S. enterica, E. coli* etc.)

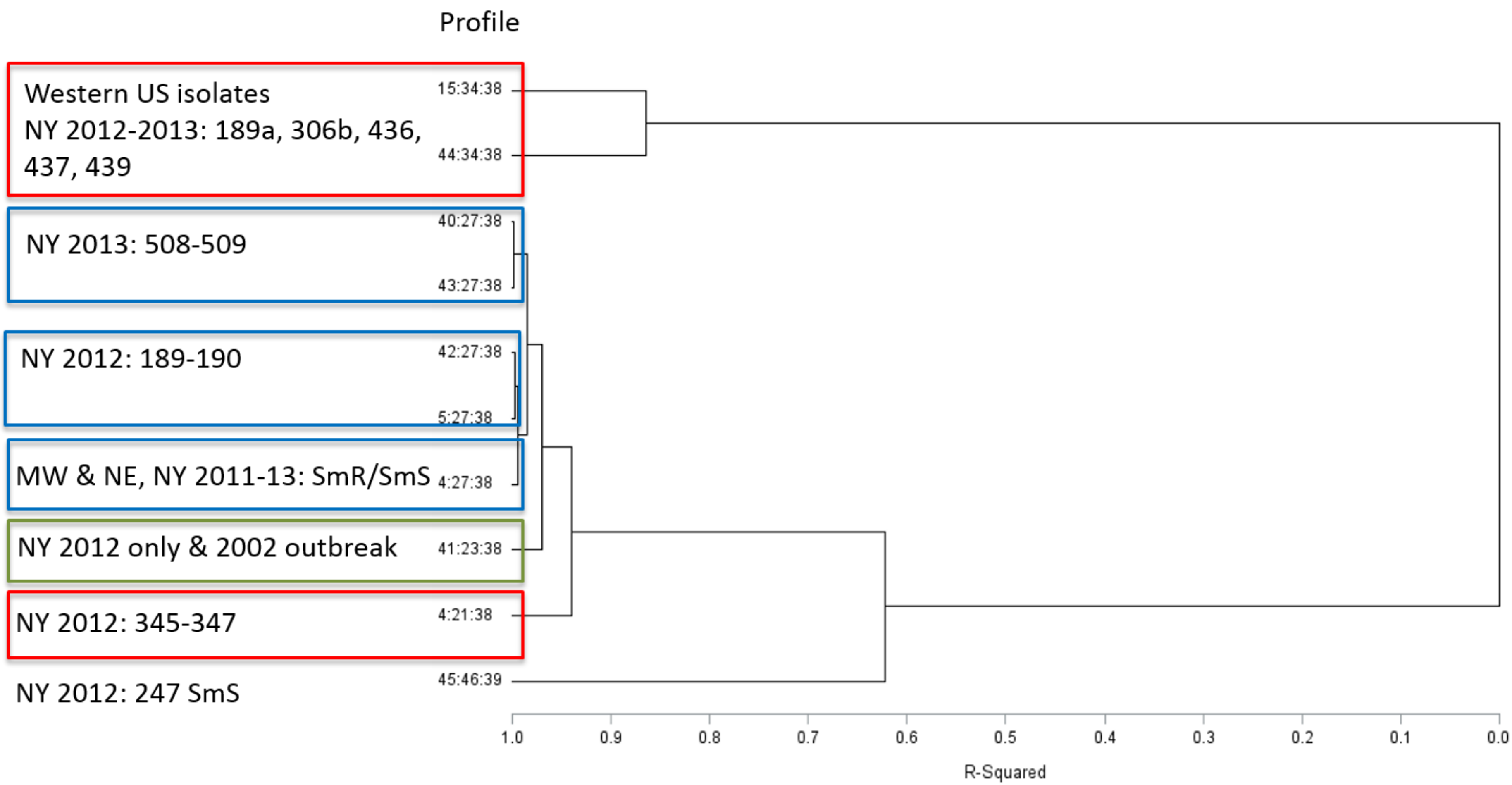
Origin of strep resistance

- McGhee and Sundin (2012): Distinctly group 85 isolates based on region of origin (North America (east and west), Europe, New Zealand, and Middle east)
- Phylogenetic relationships between Sm^R and Sm^S Michigan isolates to investigate parental strains of new streptomycin resistant isolates
- Hypothesis: CRISPR spacer array pattern analysis will allow us to form relationships between NY isolates and other isolates collected world-wide.

Origin of strep resistance



Origin of strep resistance



- Green: Original 2002 introduction & Blue originates from those
- Red: arose on farm or introduced with Sm^S isolates

Origins of resistance summary

- Many unique CRISPR patterns/spacers found in NY: Diversity
- Many Sm^R NY isolates have the same CRISPR profile as the original introduction isolates NY1 & NY2: Failure of eradication and spread of resistance or unknown introductions in 2002
- CRISPR profile for some Sm^R isolates is found in Sm^S isolates at same site: Resistance development in NY or double introduction?
- Western grouping isolates and presence of rpsL mutation: New introduction?

Outline

- Streptomycin resistance update
  - History and status
  - Origin & selection
- Managing fire blight
  - Pre & post-season
  - Bloom
  - Post-bloom & Summer

Managing fire blight

- Post season: Clean up inoculum to reduce spread within and between trees
  - Prune out strikes and small cankers:

Managing fire blight

- Pre season: Clean up inoculum to reduce spread within and between trees
  - Scout and prune out oozing cankers:
    - Large - depressed discolored cracked bark: main scaffolds can't prune
    - Small – blossom & shoot infections, summer pruning cuts: numerous & hard to see/find

Managing fire blight

- Pre season
  - Apply full rate of copper at silver/green tip
    - Warm weather causes cankers to ooze > fire flight inoculum increases greatly
- Bloom (had or have history of fire blight)
  - Watch for CCE alerts and disease model forecasts for fire blight infection periods (NEWA & MaryBlyt 7.1)
  - Since **SmR Ea** has **not** been confirmed in eastern NY, use highest rate of strep for each forecast infection

Managing fire blight

- Bloom
  - Concerned about effectiveness of strep, use highest rate of Kasumin 2L at the 2nd or 3rd forecast infection
- Bloom (Organic with susceptible varieties)
  - No antibiotics (Oct 20, 2014), Highest rate of Double Nickel with Cueva, Badge X2 with hydrated lime, Serenade Optimum, or Blossom Protect
  - Run MaryBlyt 7.1 with 60-75% efficiency with forecast data to plan spray interval – use local data

Managing fire blight

- Additional bactericide considerations
  - Streptomycin: locally systemic & Oxytetracycline & Kasugamycin protectants

- Post-Bloom & Summer: Copper (protectant)
    - Can cause fruit russet: not a concern in nursery or during establishment - survival
    - Apply with adequate drying time
    - Protectant: reduces surface bacteria
    - Terminals can outgrow protective residues of copper
    - Low rate fixed copper program: 7-10 day schedule until terminal bud set

Managing fire blight

- Post-Bloom & Summer: Apogee
  - Retards vigorous shoot growth in young trees & is best protection against shoot blight
  - Make two applications: 6-12 oz/100 gal (3-6 oz/100 gal for tree <5 years) beginning at 1-3” shoot growth & 14-21 days later

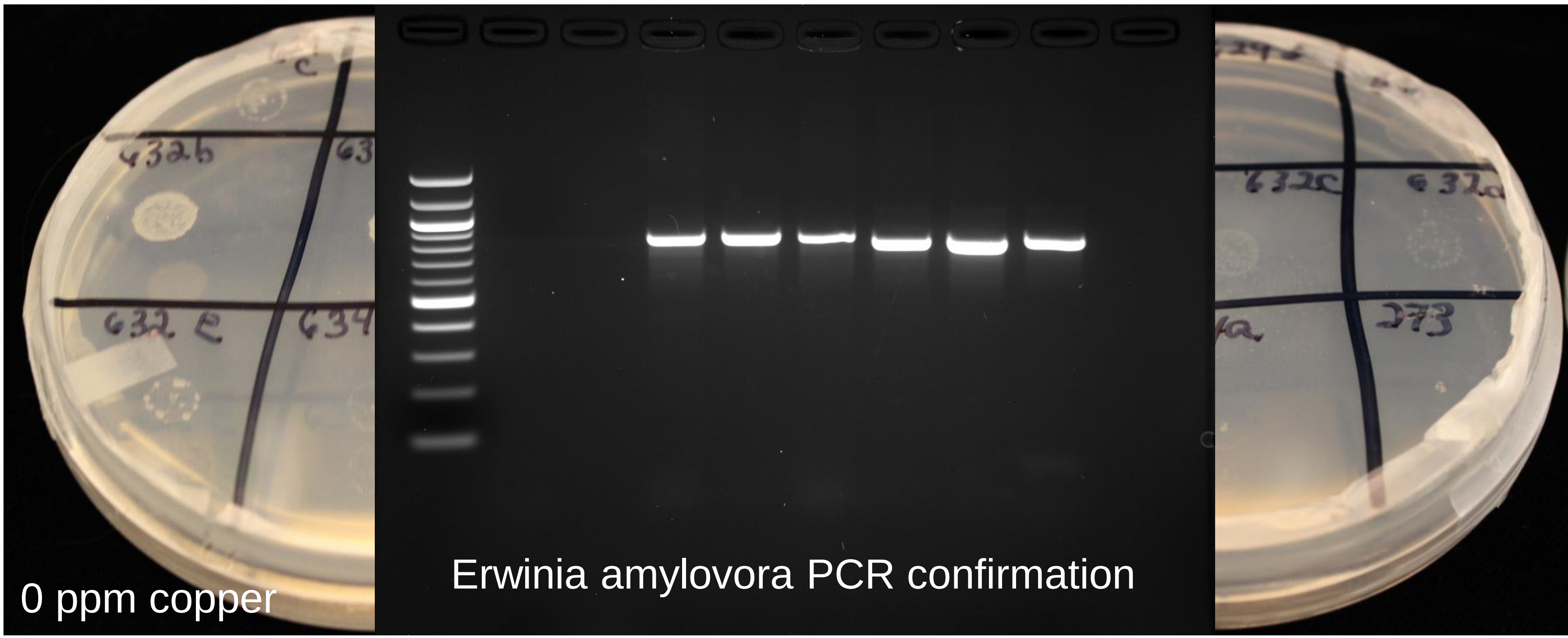
Managing fire blight

- Post-Bloom & Summer: Pruning newly developed strikes
  - Remove as soon as noticed
  - Prune on a cool dry day
  - Cut into last season's growth - At least 12” into healthy tissue
  - Young trees: if 12” is into the main scaffold – remove/replant

Acknowledgments

- State, federal, and institutional funds appropriated to the New York State Agricultural Experiment Station
- Funding support by the NYS Apple Research and Development Program
- New York State Department of Agriculture & Markets - Specialty Crop Block Grant
- Summer Crew!

Questions?


0 ppm copper
Erwinia amylovora PCR confirmation
632b
632c
273