Insect Vectored Bacteriology and Virology

Foreign Disease-Weed Science Research Unit, USDA-ARS, Ft. Detrick, Frederick, Maryland

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Foreign Disease - Weed Science RU

- BSL-3 plant pathogen containment facility
  - 7500 sq ft greenhouse
  - 1000 sq ft attached lab
- Separate office, lab, and propagation greenhouse complex with two BSL-2 labs approved for Select Agents and an Insectary Greenhouse

Bacteriology and Virology Projects

- Plum Pox virus (PPV) in stone fruit (reservoir hosts; vernalization)
- *Xylella fastidiosa*: genomics, transcriptomics, and proteomics (with Paul Stodghill, USDA-ARS Ithaca NY)
- *Ca.* Liberibacter asiaticus: genomics and phloem gene expression in infected citrus
- *Rathayibacter toxicus* and related species:
  - Genomics and biology
  - Diagnostics (with Doug Luster, USDA-ARS Ft. Detrick MD)

Canine PPV Patrol

- Training site for detector dogs (Tim Gottwald and Gavin Poole, USDA-ARS Ft. Pierce Florida)
- Detect PPV as soon as two weeks after inoculation and in infected budwood

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Why does anyone care about *Rathayibacter toxicus?*

- Produces tunicamycin and causes Annual Rye Grass Toxicity (ARGT)
- Found only in Australia (so far…)
- Caused $40 million in damages in 2010 in western Australia

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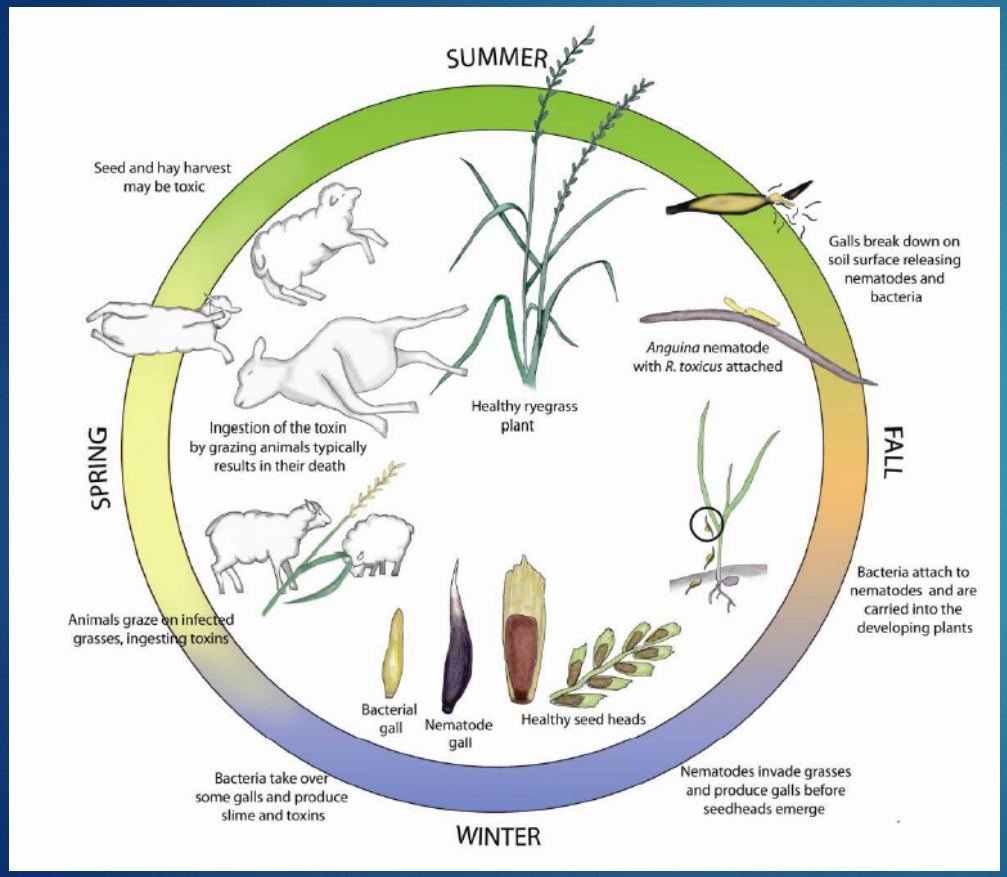
- USDA-APHIS Select agent

Rathayibacter Disease Cycle



- Associated with *Anguina* or related nematodes (dictates host range)
- Plant pathogen??? Nematode pathogen???
- Why is toxin made?
- Is the TGC mobile?
- How is toxin production regulated?

From "Recovery Plan for Rathayibacter Poisoning" TD Murray et al, 2014

Many *Rathayibacter* species known

- World-wide distribution
- Environmentally hardy
- Described species:
  - *R. toxicus* is known to produce toxin
  - *R. agropyri, R. woodii, R. iranicus* encode TGC but production unknown
  - Others don't (*R. tritici, R. rathayi, R.*

029-P1: Discovery of tunicamycin-related gene clusters in three *Rathayibacter* species, including one endemic to the Northwest US (*R. agropyri*)

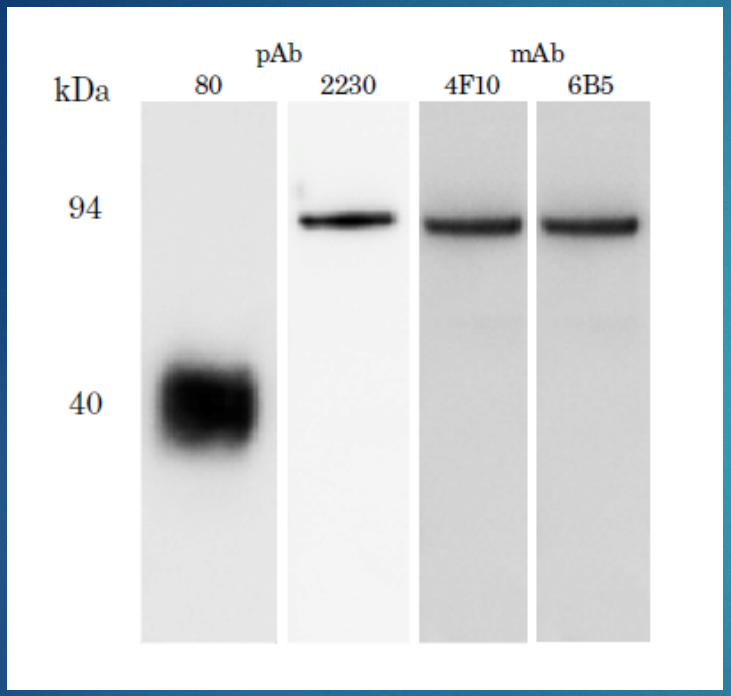
R. toxicus is a threat to US agriculture: potential nematode vectors are already in US

- *Anguina agrostis*: bentgrass seed gall nematode
- *A. pacificae*: pacific shoot gall nematode
- *A. tritici*: wheat seed gall nematode
  - Not reported in US since 1975
- *A. agropyronifloris* on western wheatgrass (*Agropyron smithii*)

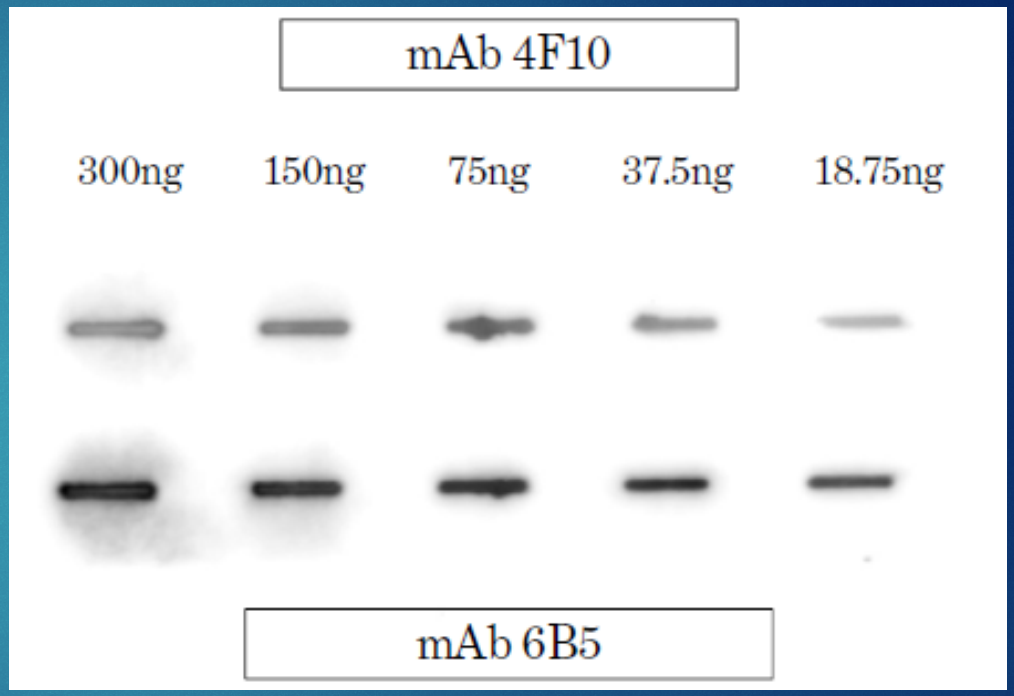
Rathayibacter Projects

- Transformation system so we can:
  - knock out TGC
  - tag with fluorescent protein
- Agglutination tests with various *Rathayibacters* and nematodes
- Surveys of Northwest US (with Tim Murray, WSU and Brenda Schroeder, U of Idaho)

Antibodies for *R. toxicus* Diagnostics



Western blot with total protein



Slotblot with protein from cell-free growth supernatant

Doug Luster and Mike McMahon

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