

What Lies Beneath:
Exploring the Soilborne
Microbial Complex of Prunus
Replant Disease

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A little bit about me...



- From Southeastern Indiana
- Raised on an apple and peach orchard
- Undergraduate studies at Purdue University in Plant Biology
- Completed my Master of Science in Plant Pathology at UC Davis under Greg Browne

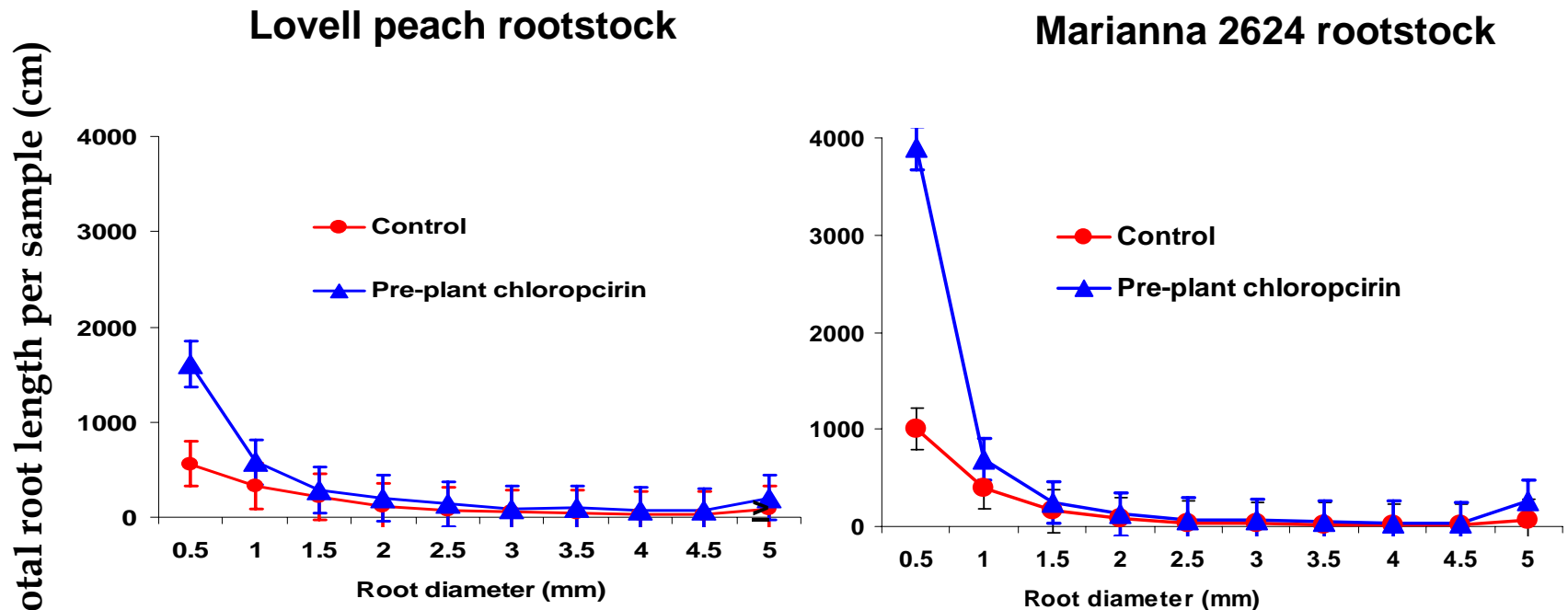
Prunus Replant Problems

- Heavily researched, starting in the 1940's
- Symptoms appear to be universal
 - Stunted shoot growth
 - Loss of fine feeder roots
- Identified a wide variety of causalities
 - Abiotic
 - Biotic
- Disease control through fumigation



Healthy almond tree (L) vs. PRD-affected tree (R)

Prunus Replant Problems



(Root length densities, as determined by root excavation and digital imaging)

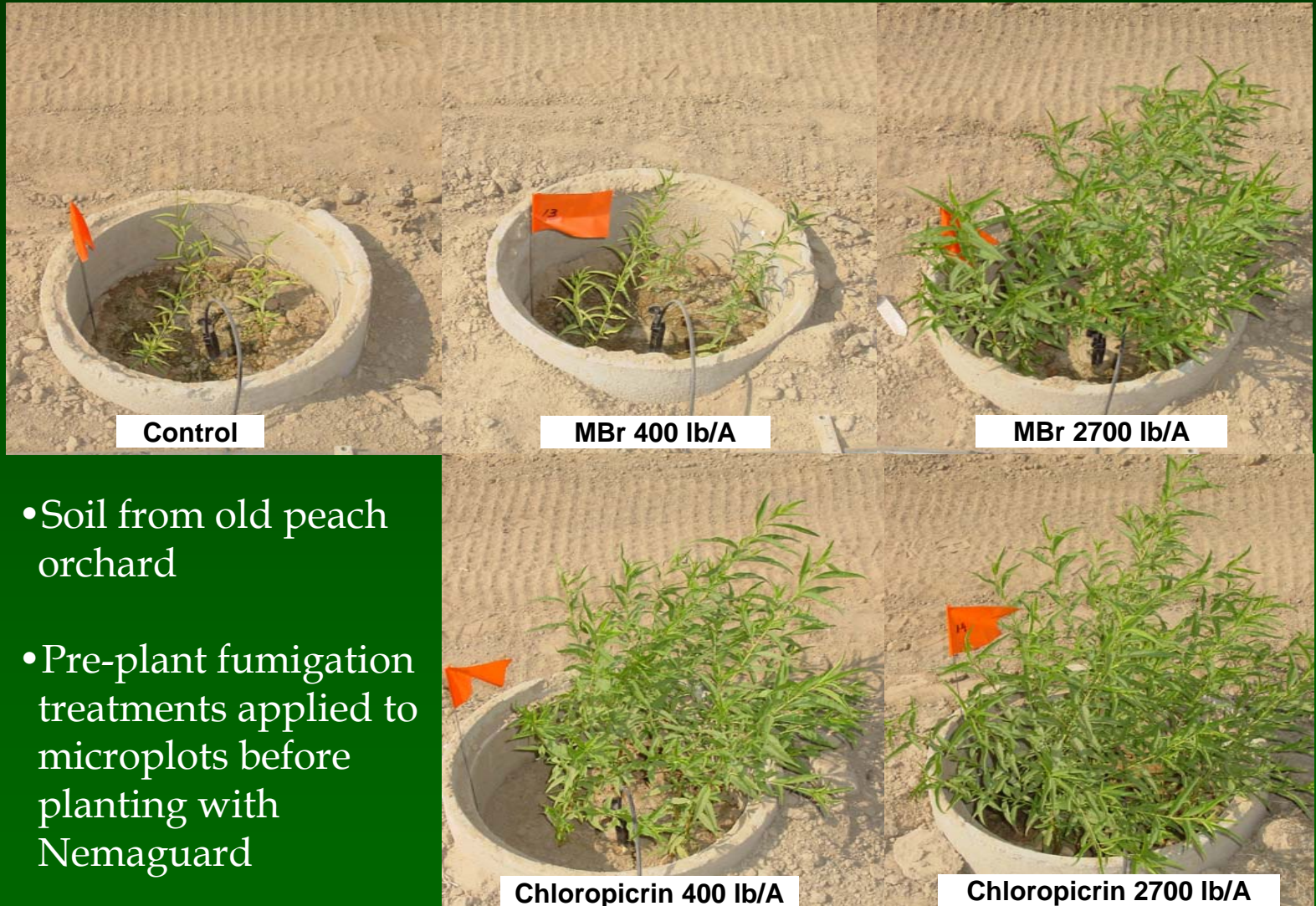
Durham, CA, 2004

Biological Mediated Replant Problems of Prunus

- **Plant parasitic nematodes** (ring, lesion, root knot), approx. 35% of almond and fresh stone fruit acreage, 60% of cling peach acreage infested (McKenry)
- **Aggressive pathogens** (i.e., *Phytophthora*, *Armillaria*, *Verticillium* spp.)
- **Prunus replant disease (PRD)**: incidence nearly universal in Prunus planted after Prunus; severity varies greatly (Browne)



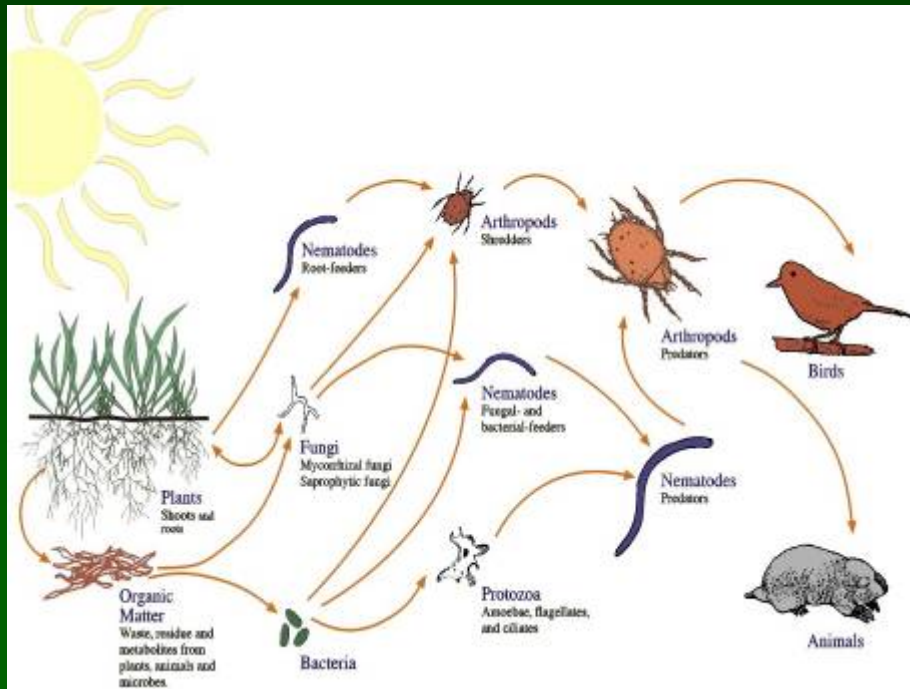
Control of Prunus Replant Disease



- Soil from old peach orchard
- Pre-plant fumigation treatments applied to microplots before planting with Nemaguard

At end of first growing season, Nemaguard peach seedlings

The Soilborne Microbial Community: What is that?



- Soil provides a diverse habitat
 - Arthropods
 - Bacteria
 - Fungi
 - Nematodes
 - Protozoa
 - Viruses
- Each member serves a purpose

The Soilborne Microbial Community: Affecting the way Plants Grow



- Conditions determine community shifts
 - Environmental
 - Plant Hosts
 - Community members and substrate present
- Communities have demonstrated the ability to cause beneficial and negative effects on plant growth.

Examining Etiology of PRD



- Microbial agents that may cause PRD:
 - Protozoa
 - Nematodes
 - Fungi
 - Bacteria
 - Arthropods
 - Viruses

Examining Etiology of PRD



Roots and soil from healthy and RD-affected trees

Whole-organism assays

- Direct quantification (nematodes)
- Isolation and quantification in culture (fungi, bacteria)

Pathogenicity tests

Molecular assays

- DNA fingerprinting (PCR of rDNA; fungi, bacteria,
- cloning of fragments, DNA-sequence-based id)

Soil treatments

- Semi-selective chemicals or treatments in Microplot, GH tests

Nematode involvement in PRD

- **Butte County orchard RD trials (2001-2004):**
 - No significant counts of plant parasitic nematodes (3 orchards)
 - Sugar floatation and mist chamber extractions
- **Parlier microplot trials (2002-2005):**
 - Only pin nematode (*Paratylenchus* sp.) present in significant number (3 repeated trials); not correlated with RD incidence
- **Madera County orchard trials (2003-):**
 - To date, minor or no nematode involvement; sampling continuing
- **Conclusion:**
 - RD and nematode parasitism not the same, they are distinct replant problems
 - Associated with biological agent(s) other than plant parasitic nematodes

Examining Etiology of PRD



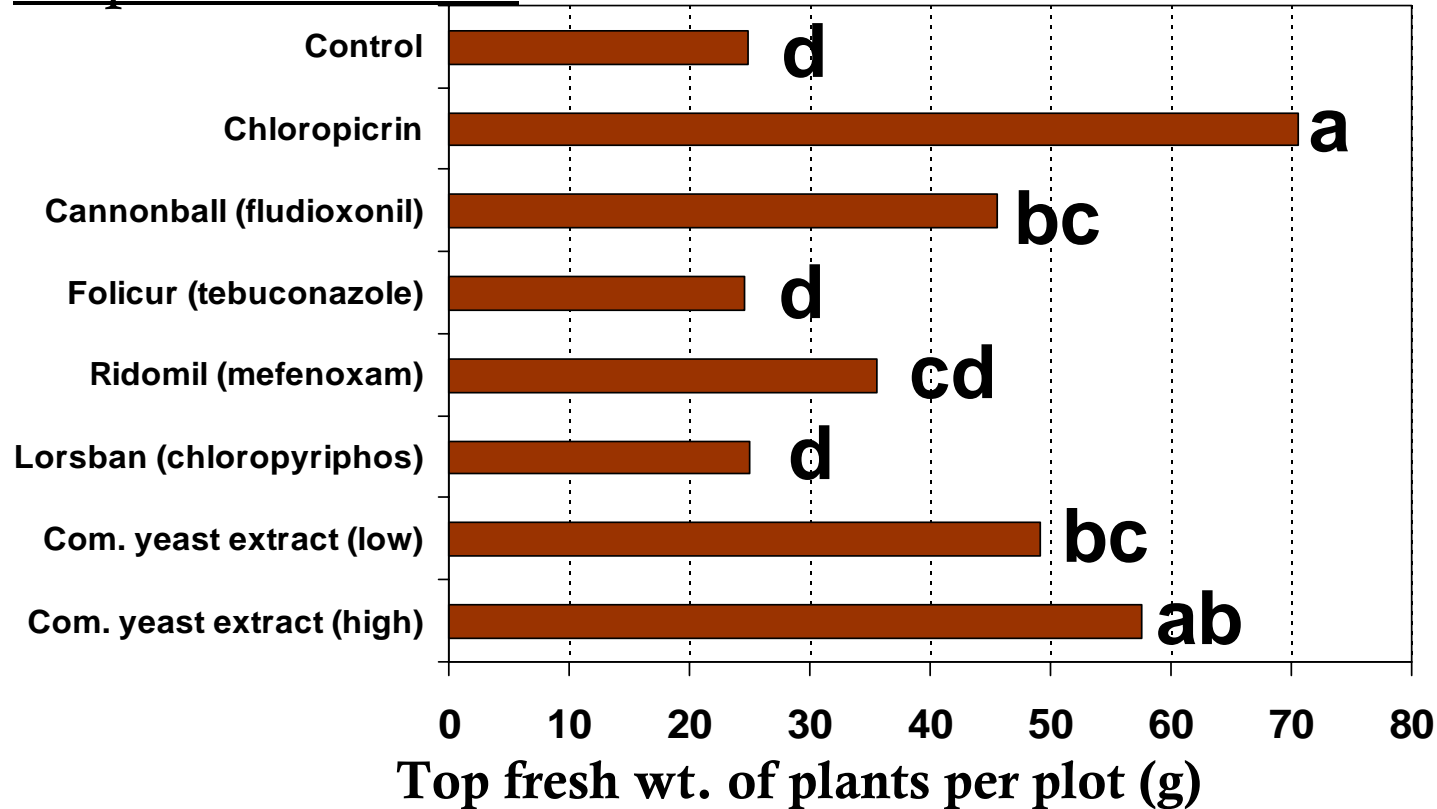
- Microbial agents that may cause PRD:
 - Nematodes
 - Fungi
 - Bacteria
 - Arthropods

Determining effects of semi-selective soil treatments on severity of PRD



Effect of pre-plant semi-selective treatments on the growth of replanted Nemaguard peach

Preplant treatment



6 Replicates in CRD, One trial year

Examining Etiology of PRD



- Microbial agents that may cause PRD:

- Fungi
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Examining Etiology of PRD



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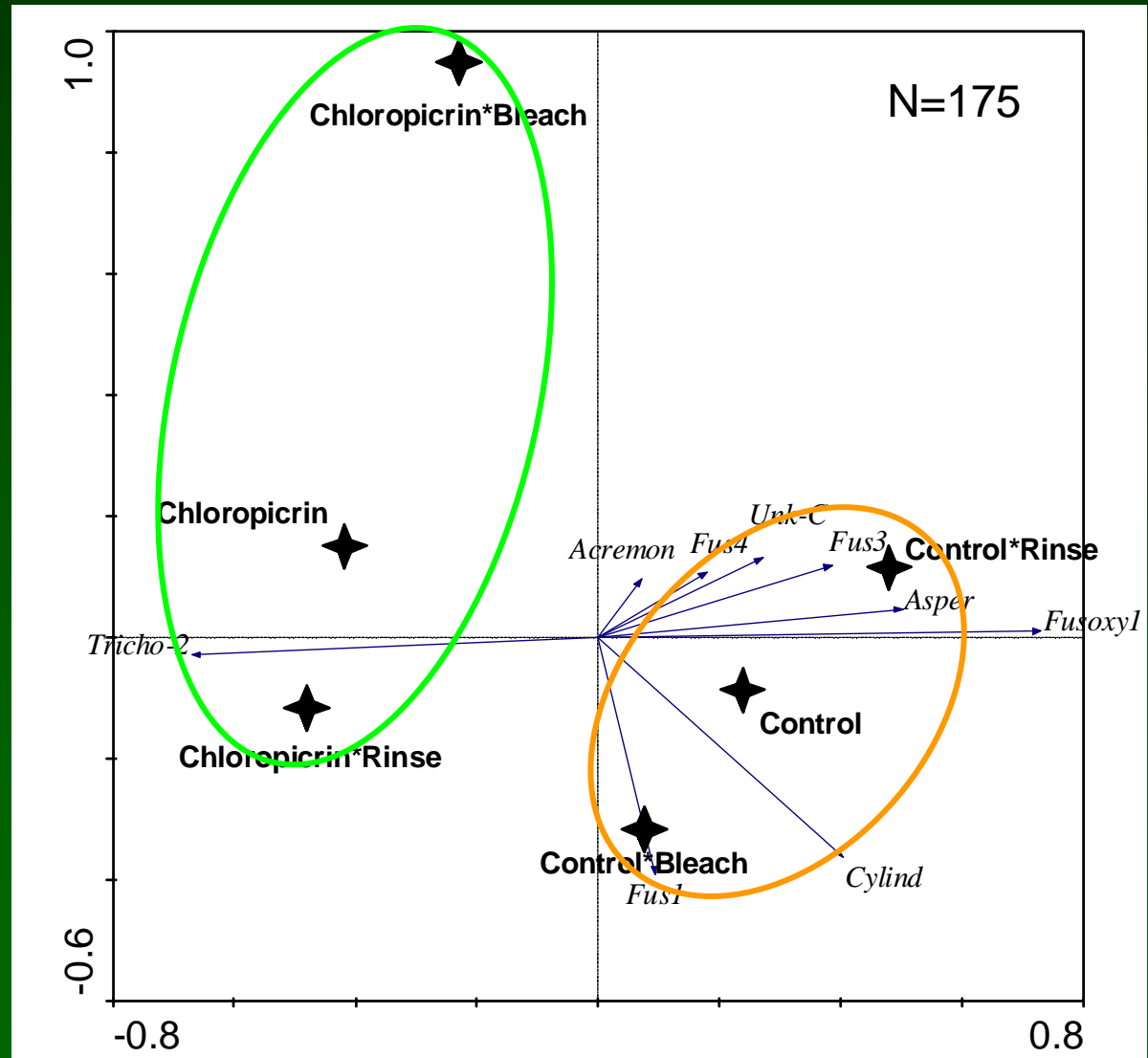
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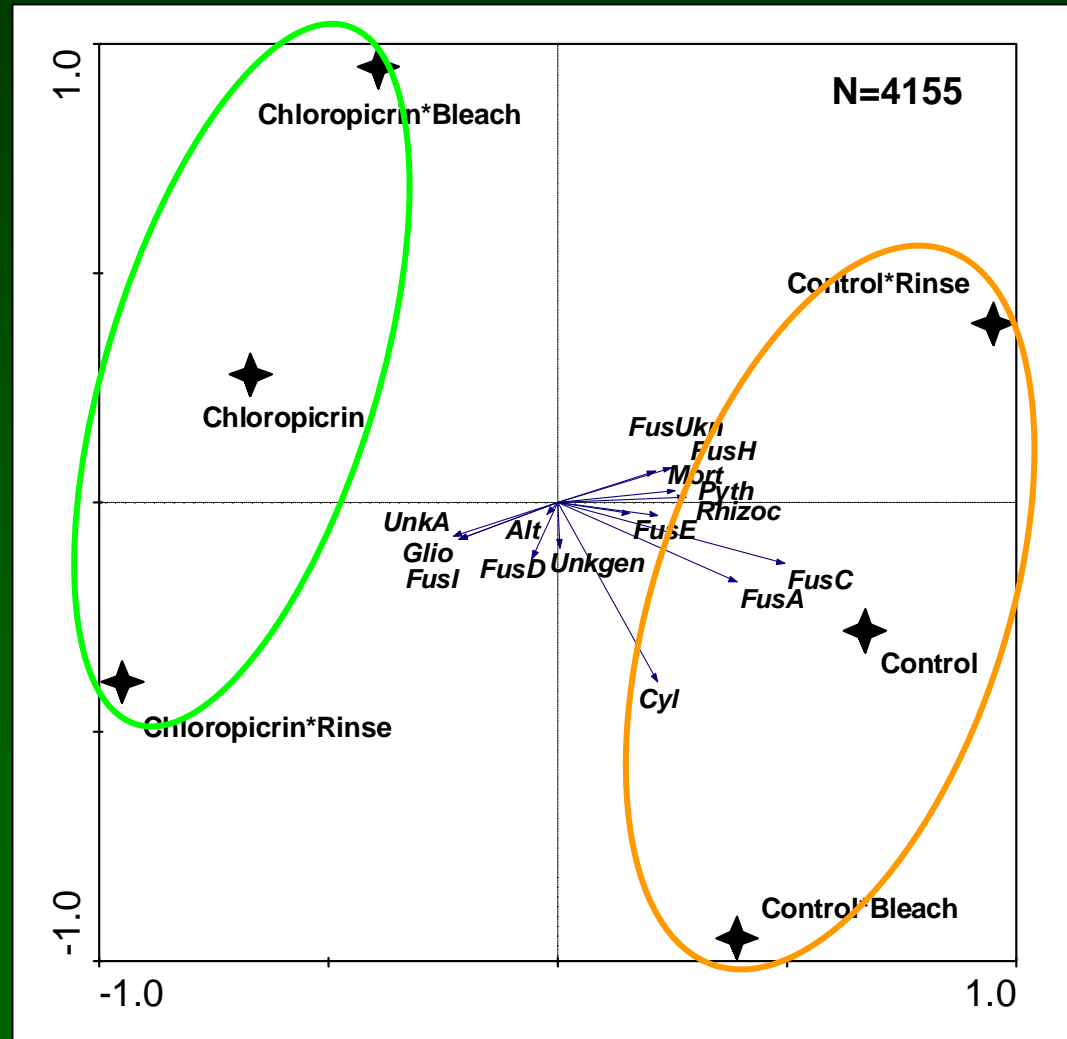
Examination of fungal microbial community shifts involved in PRD.

- Redundancy analysis of fungal communities associated with PRD using culture based technique, at right are shown shifts in fungal community in the 2007 Firebaugh trial.

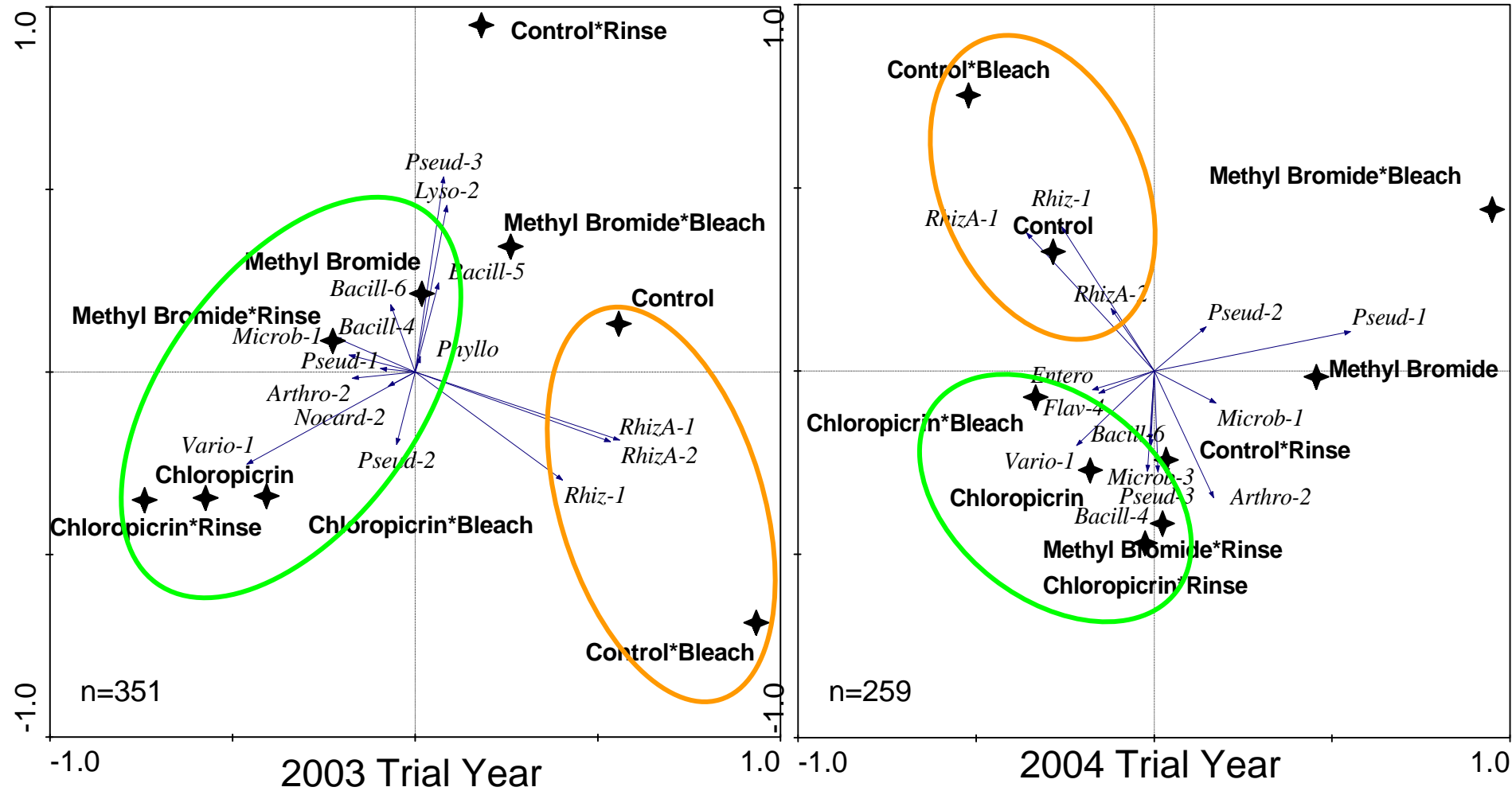


Examination of fungal microbial community shifts involved in PRD.

- Redundancy analysis of fungal communities associated with PRD using culture based technique, at right are shown shifts in fungal community in the Durham 2004 trial.



Examination of bacterial microbial community shifts involved in PRD.



Redundancy analysis of bacterial communities associated with PRD using culture based technique, at right are shown shifts in fungal community in the Parlier 2003 and 2004 trial.

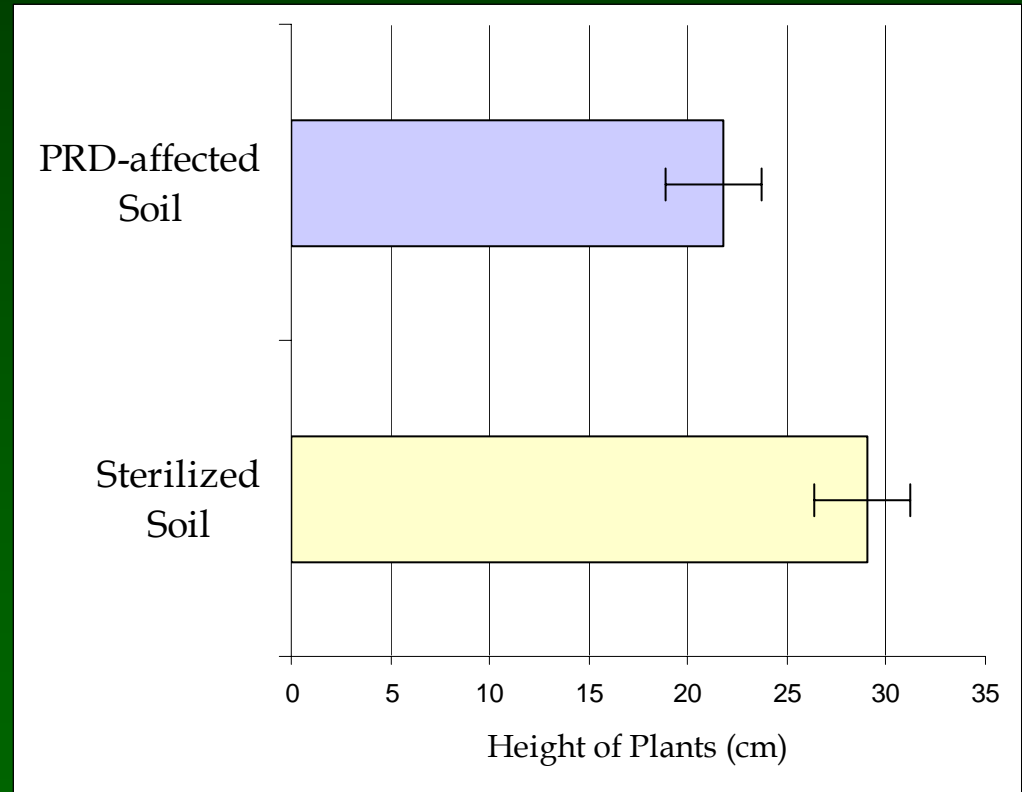
Greenhouse Experiments: Application of Associated Bacteria



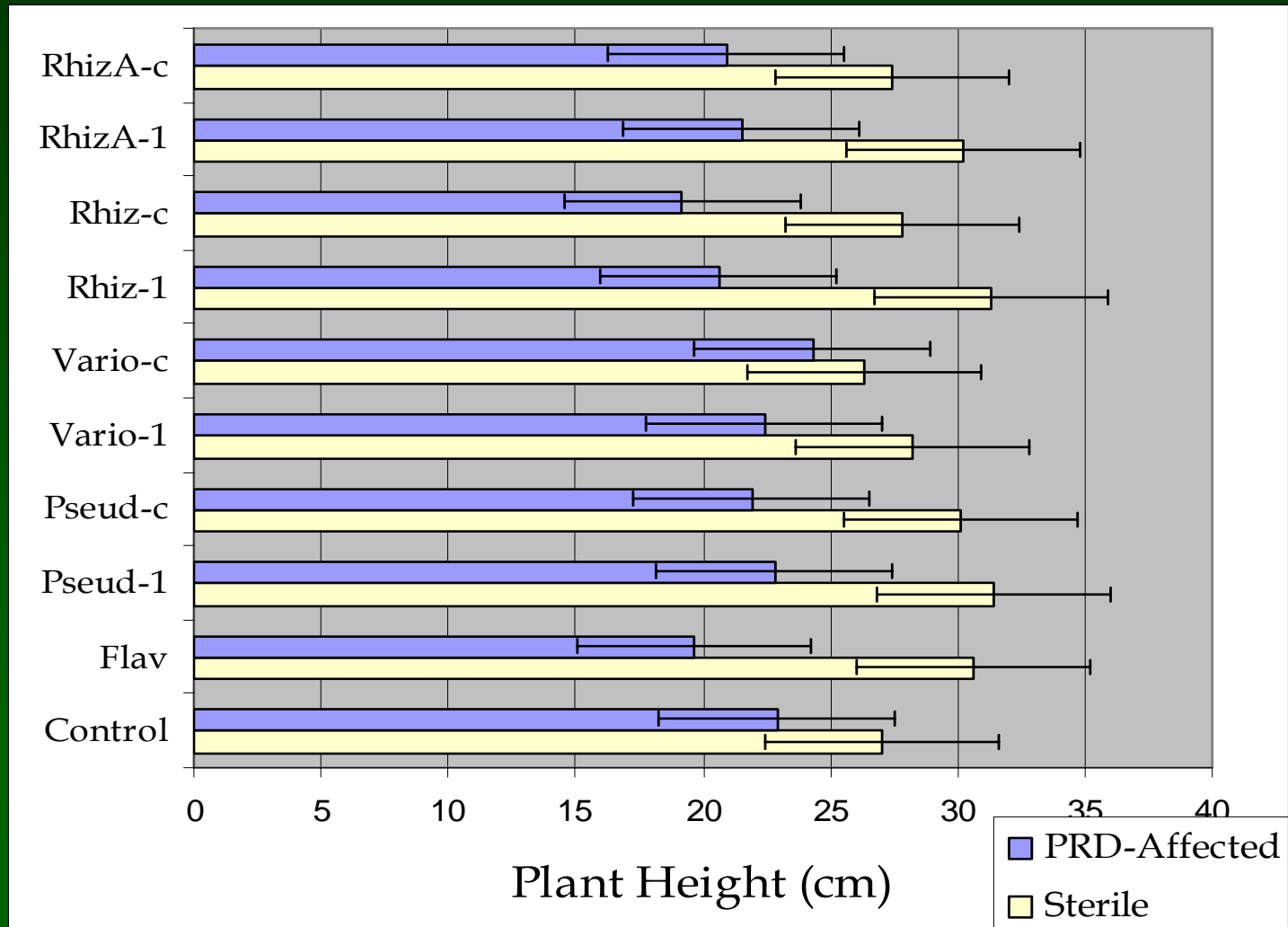
Bacterial isolates were applied to sterilize and PRD-affected soil.

Nemaguard peach seedlings were planted immediately after application of bacteria.

Greenhouse Experiments: Disease Expression in the Greenhouse

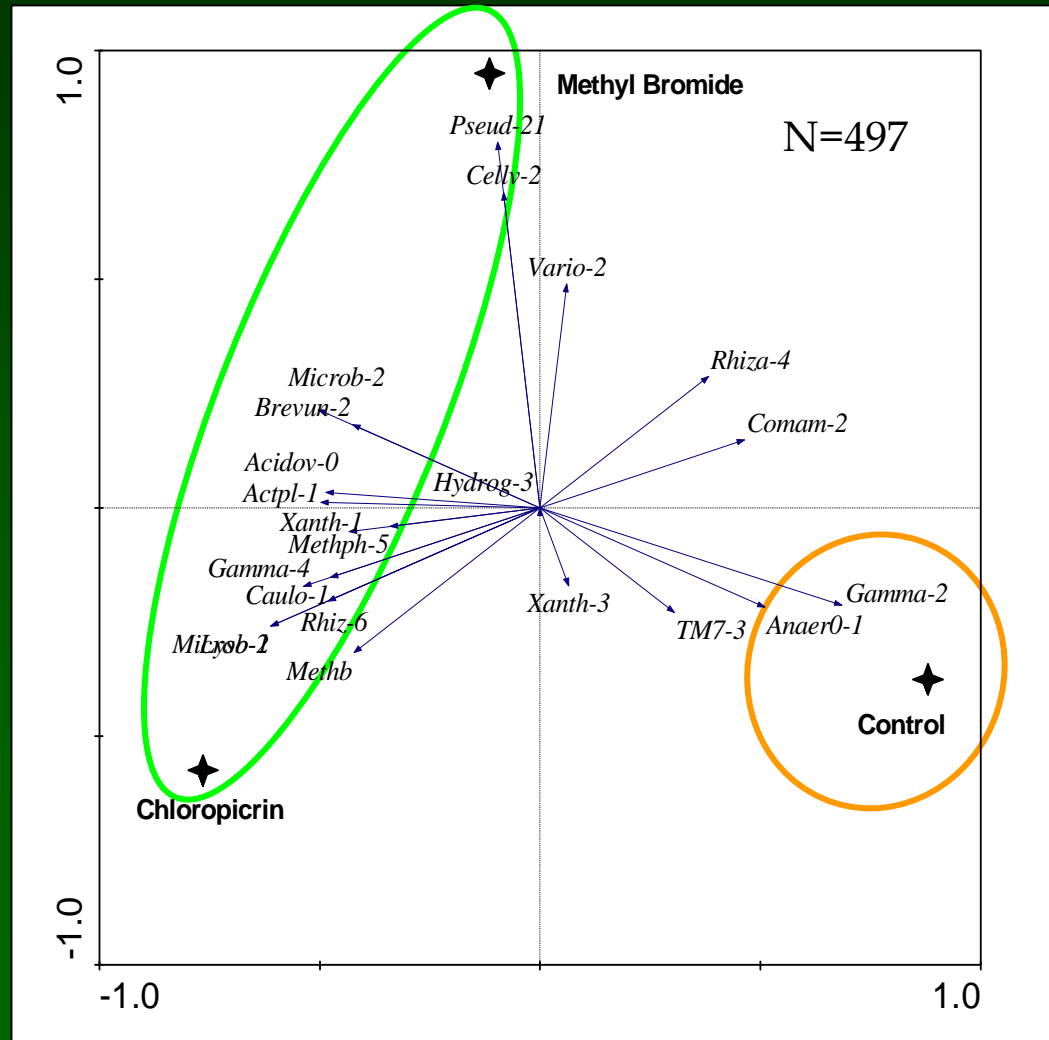


Greenhouse Experiments: Application of Associated Bacteria



Examination of Culture Independent Bacterial community shifts involved in PRD.

- Redundancy analysis of culture independent bacterial communities associated with PRD using culture based technique, at right are shown shifts in bacterial community in the Parlier 2003 trial.



Examining Etiology of PRD



- Microbial agents that may cause PRD:
 - Fungi
 - Bacteria

Conclusions

- Replant disease (RD) of almond is a biologically induced disease showing poor growth or survival in almond planted after *Prunus*.
 - Prevented by pre-plant fumigation with fumigants containing Chloropicrin.
- Some fungi and bacteria are being implicated, multiple approaches are being used to determine causal agents and developing greenhouse assays.
- An unfavorable microbial community may be responsible for the disease:
 - Shifting the microbial community in favor of the newly planted *Prunus* sp. tree may provide some level of disease control.
 - Pre-plant Application of treatments or cover cropping may provide alternative control reducing the use of fumigants

Thank You!

Project involved multiple collaborations with UC, UCCE, USDA-ARS scientists, UC Faculty, Growers, Private Industry, and Funding sources.

