Microbial ecology of red raspberries under different production regimes

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Outline

• Microbial ecology basics
• Data from current experiments
• Future applications
Microbial ecology – why do we care?

- **Microbial ecology** is the *ecology* of microorganisms: their relationship with one another and with their environment. It concerns the three major domains of life—Eukaryota, Archaea, and Bacteria—as well as viruses.
- The microbiome is all the microorganisms found in association with something.
- The Human Microbiome Project was launched in 2008 to characterize our microbiomes.
- In the human microbiome, microbial cells outnumber human cells 10:1, microbial genes outnumber human genes 100:1.
Phytobiomes

- All the microbes associated with plants
- Can be subdivided by location on the plant; phylosphere, rhizosphere, and endosphere
- Fossil record shows plant and microbe associations from 460 million years ago.
Why is this important now? How has science changed to make it important?

- Science is easy – our goal is always to correlate some variable with an experimental observable.
- Technology improvements lead to advances in the experimental observable.
- You can only “observe” things you can measure.
Observing microbes on plants

- 15-30 yrs ago, we can only see one microbe at a time and we had to kill all of the others with selective plating. The results were mostly qualitative.

- 5-15 yrs ago, we started using DNA methods routinely and started to see more than one microbe at a time, and its quantitative.
Past limitations in understanding the phytobiome

Analogous to trying to understand how a city functions but only be able to count 1987 green Nissans.

This leads to a very simple model of plant pathogenesis.
Model of plant pathogenesis

We like to think in simple terms.

In reality, it's closer to the shifting allegiances in the middle east.
Current technology

Microbial sample

Extract DNA

\[ \downarrow \]

Amplify a gene common to all

Sequence, bin and compare to database

Allows us to see all microbes at a given time point.

![Bar chart showing percentages of different species: Red species, Green species, Blue species.]

- Red species
- Green species
- Blue species
The technology is not new, it's just now affordable.

I could have done the same experiments in 2008, it would have just cost 1000x more.
Now the problem is we can see all of the microbes!
Can you spot the differences?

Healthy system

Diseased system (before economic loss)
Experimental details

Compare two production regimes
1.) Control plots (no cover crop)
2.) Treatment plots (no cover crop) + 10 tons/acre equivalent composted dairy solids +1 ton/acre equivalent Mustard seed meal.

3 biological reps and 2 technical reps.

Determine Bacterial and Fungal communities for:
Spring and Fall
Bulk soil, rhizoplane and endophytes.
How many microbes in, near or on Raspberry plants?

In 120 samples, conservatively there are:

>3,500 Bacterial species
>7,800 Fungal species

Seen at least five times,
Not all species can be resolved,
Some lineages need to be targeted separately, e.g. Oomycetes
The bacteria in a raspberry field

Bulk soil
Spring 2015
control

Bulk soil
Spring 2015
treatment
The bacteria in a raspberry field

Bacteria rhizosphere and soil

Bulk soil
Fall 2015
control

Con  Trt

Bulk soil
Fall 2015
Treatment
The bacteria in a raspberry field

Spring vs Fall

Chlorobacteria, thermophiles
The bacteria the raspberry rhizosphere

Bacteria rhizosphere and soil

Con  Trt

Rhizosphere Spring 2015 control

Rhizosphere Spring 2015 Treatment
The bacteria in a raspberry field

Rhizosphere
Spring 2015

The bacteria inside or on the surface of roots

Endophytes and Rhizosphere

Cyanobacteria, known to form biofilms on roots

Inside | On the surface
Fungi in the soil

**Botrytis caroliniana**, a new species isolated from blackberry in South Carolina

**Ilyonectria macrodidmya**

Grapevine Trunk Diseases in British Columbia: Incidence and Characterization of the Fungal Pathogens Associated with Black Foot Disease of Grapevine

*J. R. Urroz-Torres, P. Haag, P. Bowen, and D. T. O'Gorman*, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia V0H 1Z0, Canada
Fungi inside and on the surface

Much greater variability in samples

Botrytis caroliniana

Co-infection by Botryosphaeriaceae and Ilyonectria spp. fungi during propagation causes decline of young grafted grapevines

M. A. Whitelaw-Weckert\textsuperscript{a}, L. Rahman\textsuperscript{a}, L. M. Appleby\textsuperscript{a}, A. Hall\textsuperscript{ab}, A. C. Clark\textsuperscript{a}, H. Waite\textsuperscript{a} and W. J. Hardie\textsuperscript{c}

Ilyonectria macrodydema
Where from here? In the short-term, we...

- Need to broaden sampling to understand how the region varies with location and grower practices.
- Need to understand the distribution of potential black foot root rot (*Ilyonectria* sp).
- Need to evaluate fields with distinct performance differences in a given season (sample the best and the worst).
- Evaluate and monitor recently established plants, to understand the microbial ecology of “replant disease”, possibly combine with different fumigation practices or other treatments.
Where from here in the longer term?

- Manage the phytobiome for agricultural benefits
- Disease and pest suppressive soils
- Biofertilizers for enhanced nutrient uptake
- Endophyte inoculums to enhance stress tolerance
- Cultivar optimization to incorporate these interactions
In case your thinking I’m some organic hippie..

Growing Profits With Microbes
Agriculture industry seizes on beneficial fungi and bacteria to help thwart disease and increase productivity

By Marc. S. Reisch

Hugh Grant, chairman of the agribusiness giant Monsanto, calls microbes “the next major platform in agriculture that will drive yield and productivity beyond the seed itself.” And seed treatments incorporating those microbes, he says, are “the biggest near-term opportunity.”
Big Ag moving into biologicals.

BAYER CROPSCIENCE ACQUIRES US-BASED BIOLOGICAL COMPANY AGRAQUEST FOR CLOSE TO US$ 500 MILLION

DEALS & DEAL MAKERS

BASF to Buy Seed-Technology Company for $1.02 Billion

Syngenta to acquire Pasteuria Bioscience

5 out of the top 6 crop protection companies have made biological based acquisitions in the last 3-4 yrs
More than “green-washing”

PONCHO®/VOTiVO™

A systemic insecticide and biological seed treatment for use on corn, and soybean, for the control of listed insect pests and listed soil plant pathogenic nematodes

ACTIVE INGREDIENTS:
Clothianidin .................................................................................................................. 40.3%
Bacillus firmus I-1582 ...................................................................................................... 8.1%

OTHER INGREDIENTS: .................................................................................................. 51.6%

TOTAL: ..........100.0%

Contains 4.17 pounds clothianidin per U.S. gallon
Contains 0.84 pounds Bacillus firmus per U.S. gallon (contains a minimum of $2 \times 10^6$ cfu/ml)

EPA Reg. No. 264-1109 
EPA Est.
What types of products will we see in the future?

The first likely products to be adopted will be mixed products or products that can be tank mixed. This will likely occur in areas of regulatory challenges or consumer preferences.

The first modes of action will be:
1. Competitive exclusion
2. Direct antagonism
3. Stress protection
Competitive exclusion

Colonizing an area free of competition is much easier.

Colonizing an occupied area is much more difficult.
Nematodes follow a prey/predator model, after nematodes appear, there is a lag phase and then nematocidal fungi appear.

Compounds or microbes that kill the pest.
Stress protection

This might be salt, temperature, water or heavy metal tolerance.

If the forecast calls for higher temperatures wouldn’t it be nice to be able to hedge your bets.
Conclusions

- This will be the “Ecology decade”, because of changes in technology.
- These new tools allows us to see all the microbes for the first time.
- Looking at the microbes in Red raspberries provides some unexpected visitors.
- *Ilyonectria* sp – Black foot root rot may be an emerging disease of concern.
- A bramble specific *Botrytis* sp may be a root pathogen of red raspberries.
- Managing the phytobiome is being embraced by BigAg crop protection co.
- Expect more biological crop protection properties.
Acknowledgements

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