Use of real-time PCR for *Erwinia amylovora* detection during bloom and potential for integration in fire blight disease forecast in Québec

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increasing issue

- Density: short branches
- Rootstock: M.9, M.26
- Varieties: Cortland, Paulared… Gala, Gingergold, Honeycrisp, Jonagold
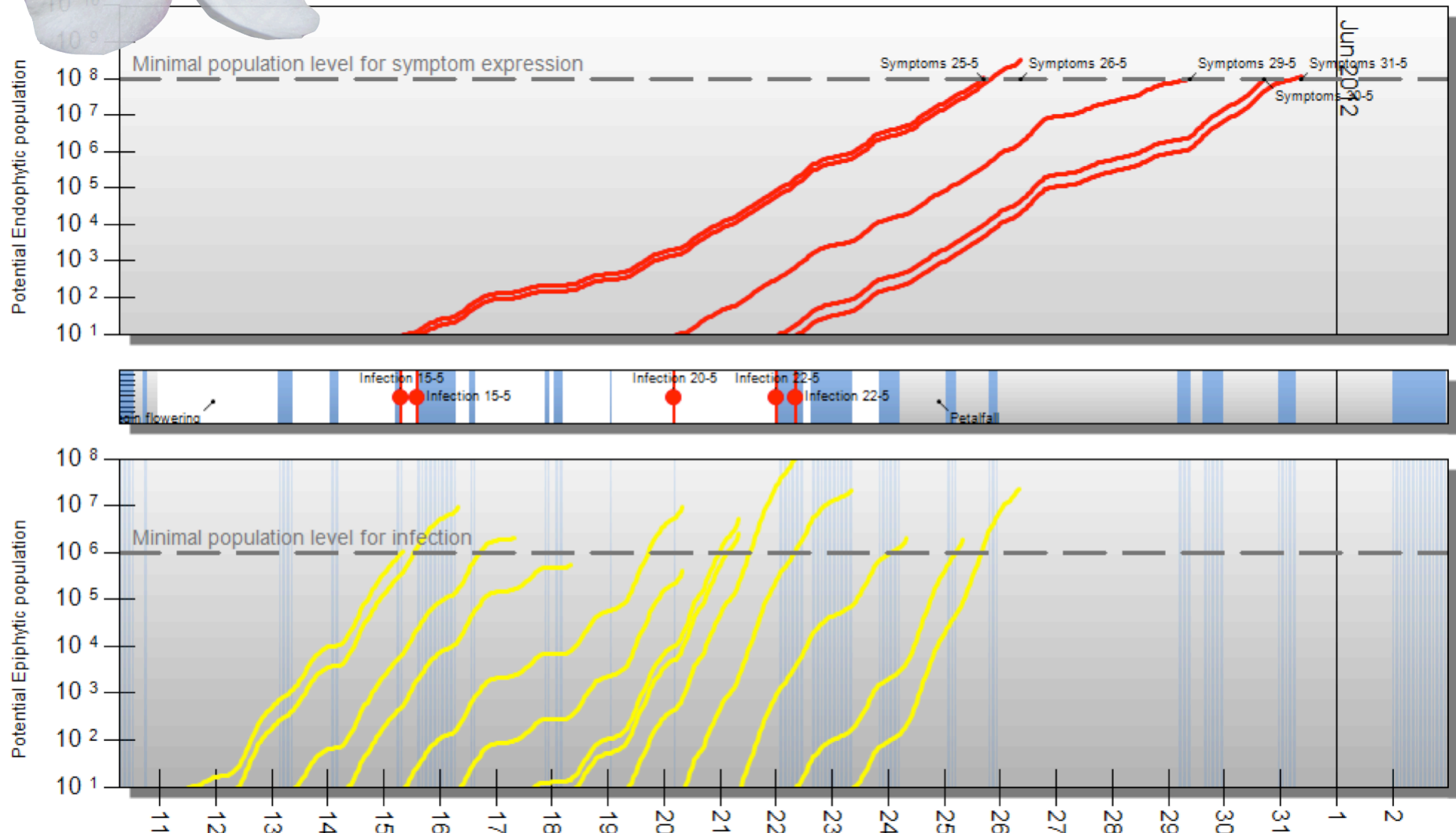- Fast initial growth
- Longer pre bloom climate…

Bacteria hasn't changed much, but orchards & climate did
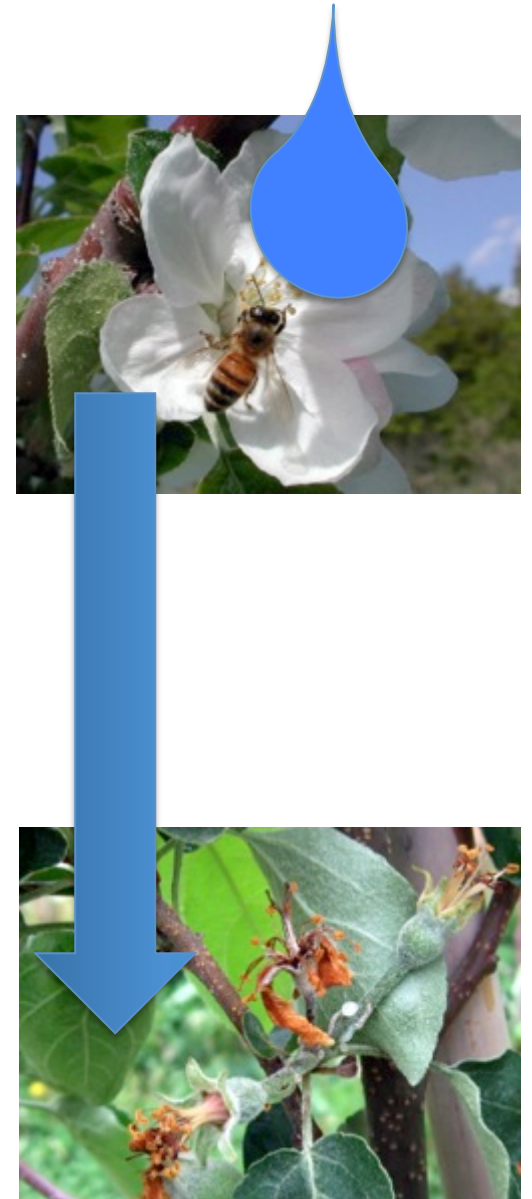
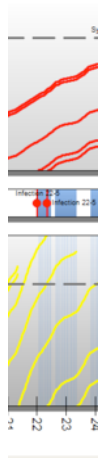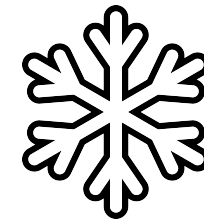# 2012 Outbreak

RIMpro Erwinia 2012

Limits of models…

- **Source of bacteria?**
- Presence of flowers
- Contamination
- Population growth
- Infection

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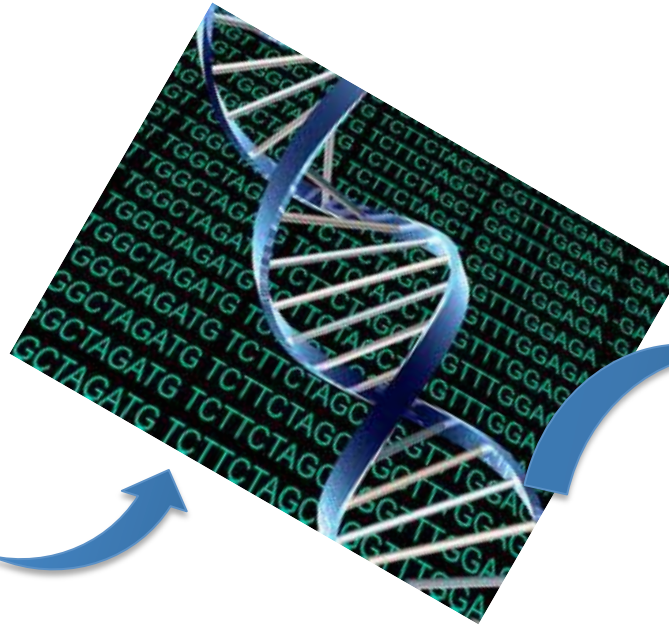# Cornered

❓ Detection ❓

- Objectives:
  - Reduce « false positive » model prognostics
  - Integrate monitoring in tactical fire blight management

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Quantifying bacterial population

• Bonus =

Antibiotic resistance monitoring

Sampling in 2012 & 2013

- Blossom = 3 collection dates
- 112 plots / 31 orchards / 5 regions
- Untreated
- $1000m^2$ per plot average
- 1 cluster / $20m^2$ (500 clusters/ha) = 0,1%
- Clusters frozen upon collection

Disease scouting

- Two visits in June
- Disease incidence

Laboratory processing off season
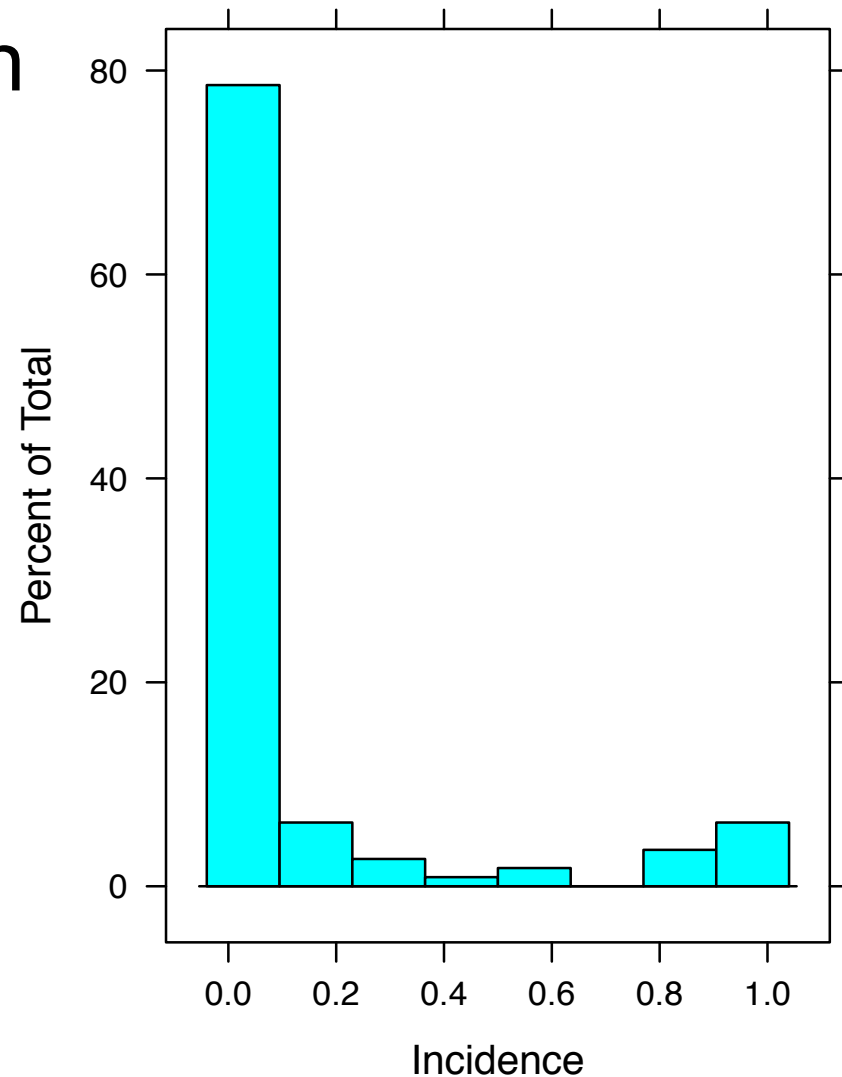
(qPCR ≠ real time detection logistics!)

qPCR assay

- No extraction
- Resuspend in 1ml TPEB (Tayler et. al. 2001)
- Vortex (10s) + sonicate (2mn) + Vortex (1mn)
- 1µl + qPCR recipe
- qPCR = chromosomal target (Gottsberger, 2010)
- 1CFU/µl = 200 CFU/clusters theoretical
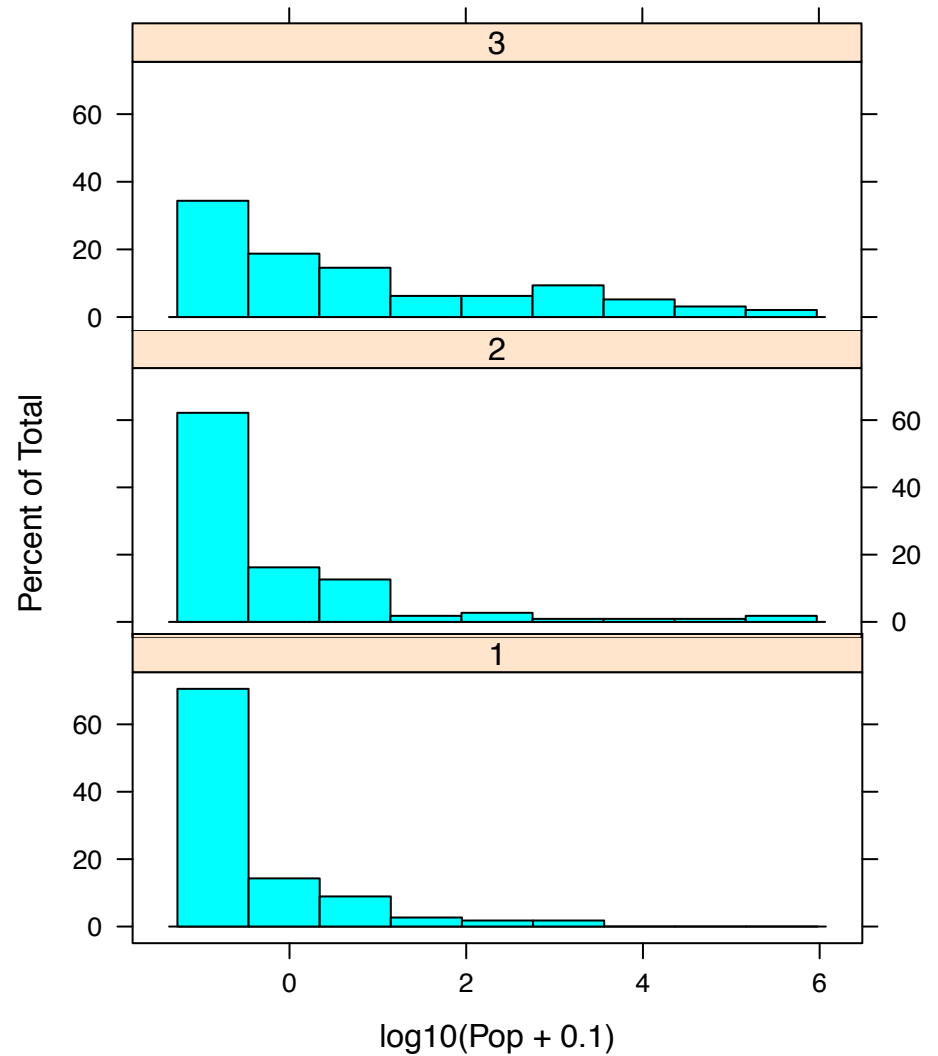- Linear with population on spiked flowers

Results

- One third of plots with disease
- 6% of plots >90% trees affected

Results

qPCR data varied
with date

log10(Pop + 0.1)

Percent of Total

Results

- Bacteria "eventually" found in 100% of orchards with disease
- Undetectable bacteria population in some clean orchards

- Clean orchards found with bacteria
- No early detection in some diseased orchards

Receiver Operating Characteristics (ROC)

- True positives (sensitivity)
- True negatives (specificity)

- False positives (bacteria, but no disease)
- False negatives (unexpected outbreak)

- Predictions with models:
  - sensitivity =100%, specificity = 0% (Cry Wolf!)
  - Two thirds of sprays useless in 2012…

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ROC Town scale (#2)

- Sensitivity = 100%

- Specificity = 33%

- Threshold: 11% positive samples
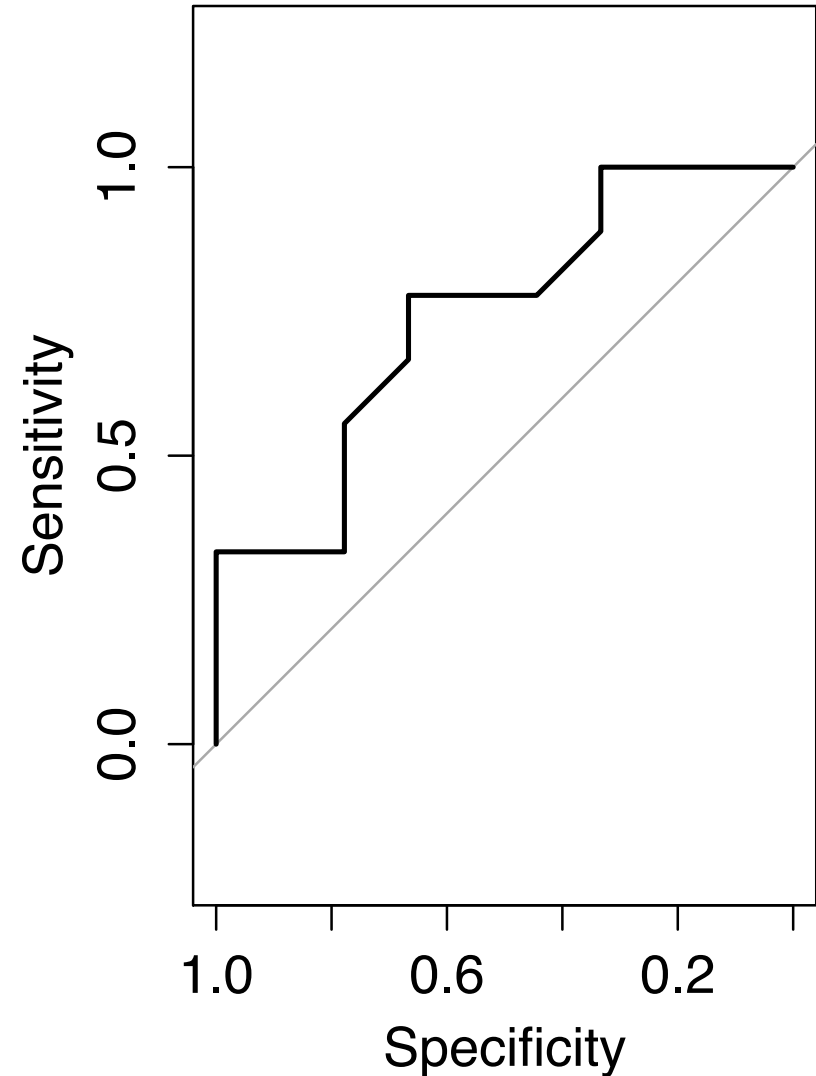
2012 summary

- 100% Sensitive = nope
- Max 15% tree incidence
- 33% specific or better
- Spray before threshold = loss of specificity

	1st		2nd	
	Positive Samples	Potential Disease	Positive Samples	Potential Disease
Plot	No threshold			
Orchard	21%	15%	23%	15%
Town	23%	15%	11%	0%
Regional	14%	0%	19%	0%

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2013 summary

- Minimal disease detected
- Almost no bacteria detected

- No bacteria in orchards with disease
- Some Bacteria in orchards without disease

Conclusion

- « potentially » useful, BUT:
  - Logistics? (real time detection)
  - Tolerance on Sensitivity…?
  - How much specificity required?
    - Geographical (Region vs Plot)
  - Cost of sampling vs
  - Cost of spray vs
  - Potential for tree loss?
  - Grower adoption?

What's next (2014-2015)

- Accumulate more cases
- Test positives for streptomycine resistance
- Distribution: plot/orchard/region
- Alternative detection techniques
  - LAMP
  - Pollen

Implementation

State run monitoring

- Selected pilot orchards
- Community approach
- Avoid major outbreaks
- Can miss some cases
- Public and/or coop ($)
- Predictable costs
- Logistics simplified
- Long term?

Private on demand

- Sampling not planned
- Private results
- Can miss major outbreaks
- Client based
- Private ($)
- Variable costs
- Offer/demand management
- Long term?

Theory meets practice

- Reliable? (ROC analysis)
- How? (Flowers vs pollen vs mummies)
- Implementation in real world? (logistics)
- Worthwhile? (spraying is cheap)

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