Sampling for grape virus diseases: Why and how?

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Grape virus disease epidemiology in QBE lab

A.J. Campbell
Red Blotch

Kari Arnold
Leafroll

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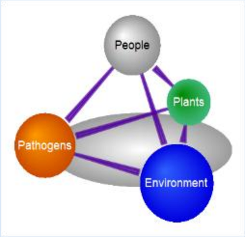
Topics for discussion

- Summary of progress
    - Leafroll epidemiology
        - Dynamics
        - Mealybug management groups
    - Red Blotch epidemiology
        - Preliminary analysis of dynamics
        - In-field Diagnostic development

The great enemy of the truth is very often not the lie, deliberate, contrived and dishonest, but the myth, persistent, persuasive and unrealistic.



QBE Lab

Leafroll doesn't spread
It's my leafroll, it's my problem

There's no problem

It's all down to infected nursery/planting stock

The problem is someone else

We don't understand the effect of leafroll on grape quality
When life gives you lemons, you might as well make lemonade

The problem is so complicated that there's nothing I can do so I'm going to pretend there's no problem and hope for the best

Between block infection causes shared costs and responsibilities

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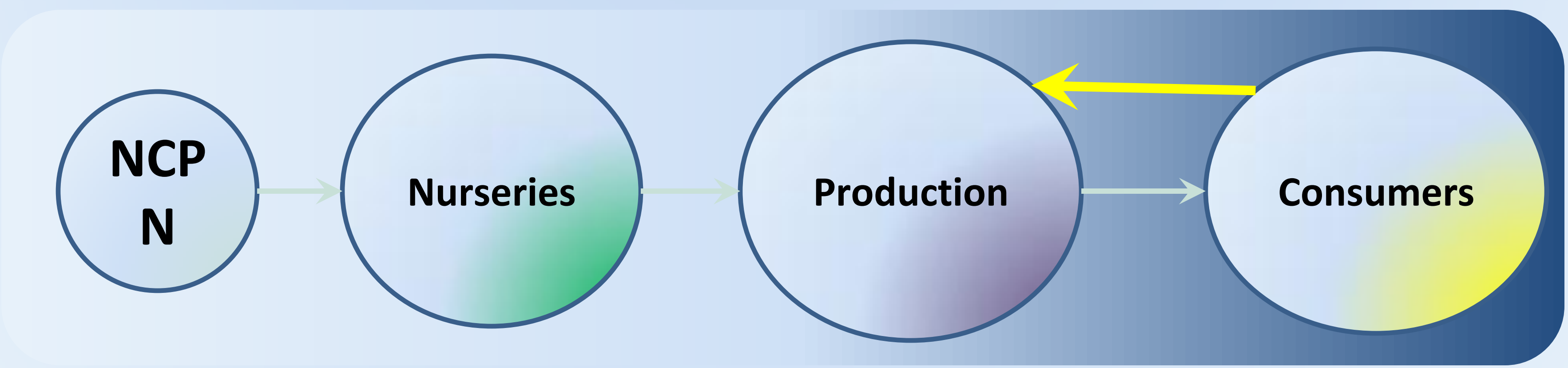

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To join a neighborhood management group

There are risks and costs to action. But they are far less than the long range risks of comfortable inaction.

Things do not happen. Things are made to happen.

Grape leaf roll disease
Grapevine leafroll associated viruses

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Grape mealybug,
Pseudococcus maritimus

Vine mealybug,
Planococcus ficus

Spatial patterns of a GLRaV epidemics

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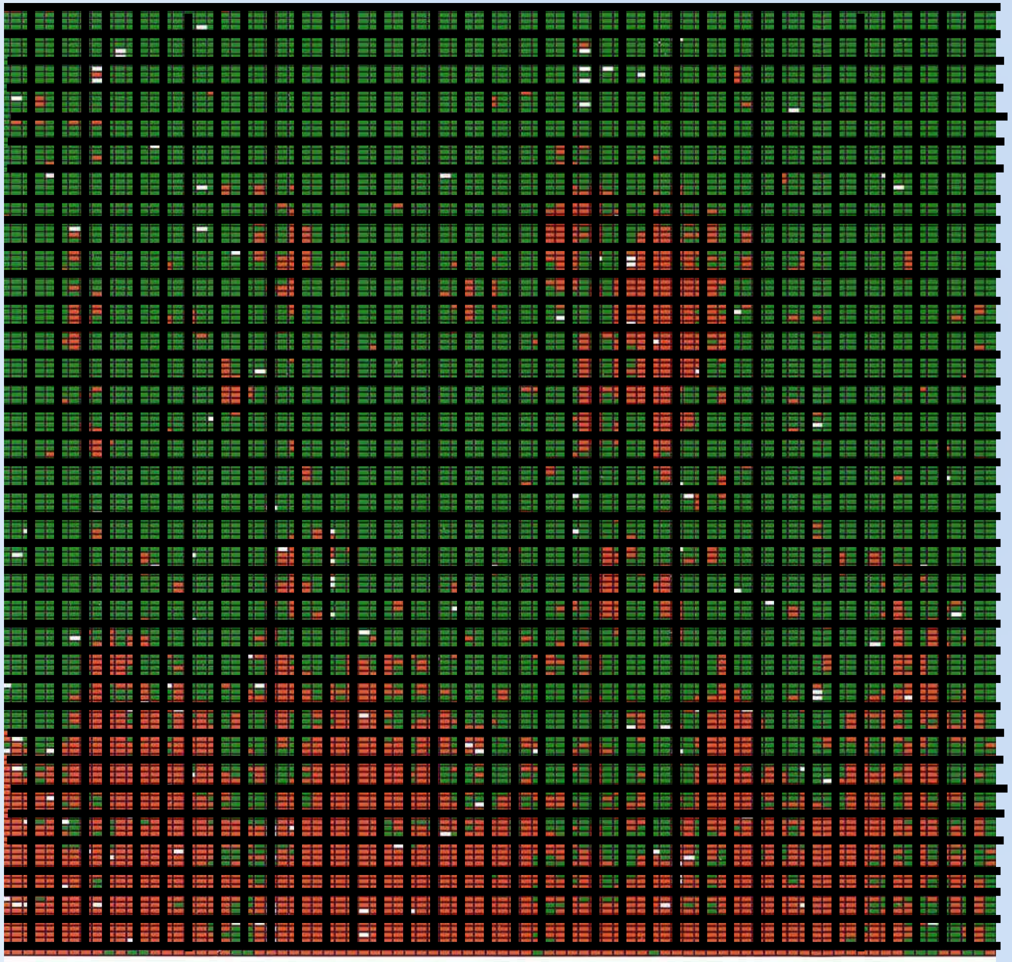
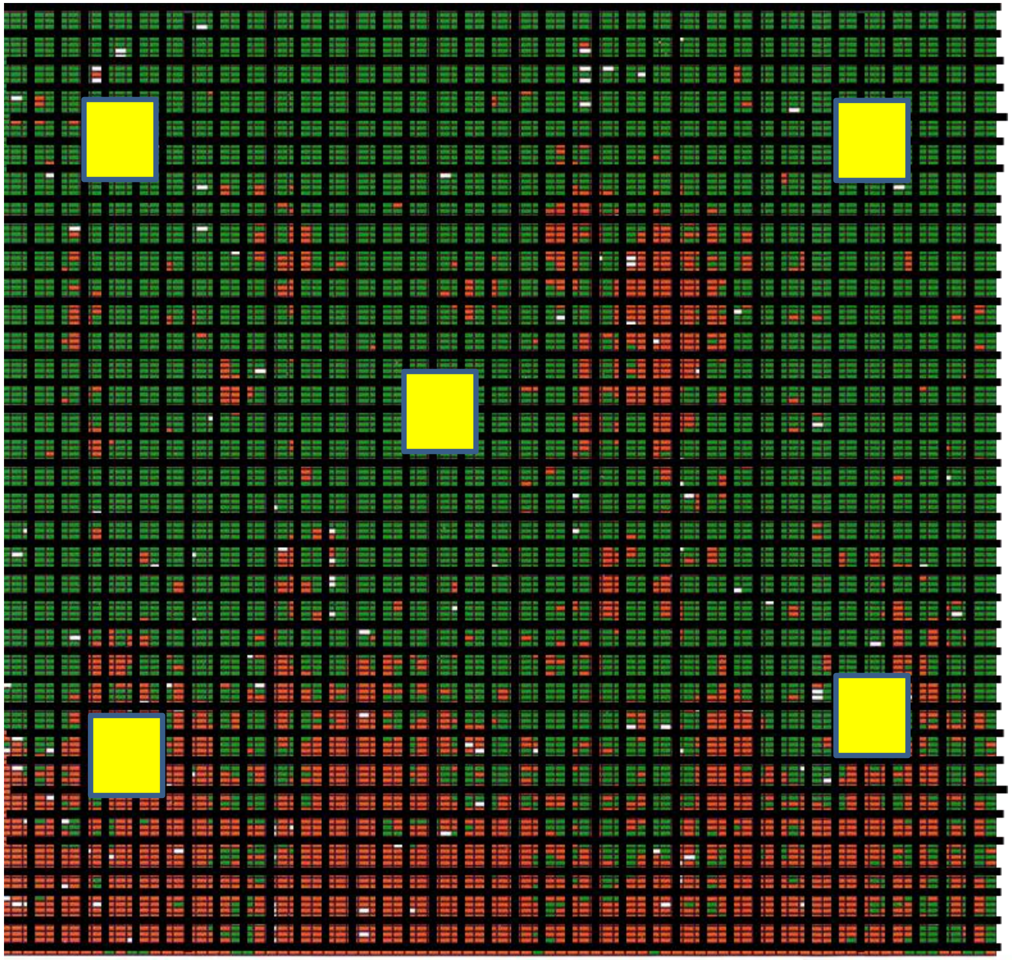


Overlay vineyard block maps with sampling grid

Count vine disease status in each quadrat.

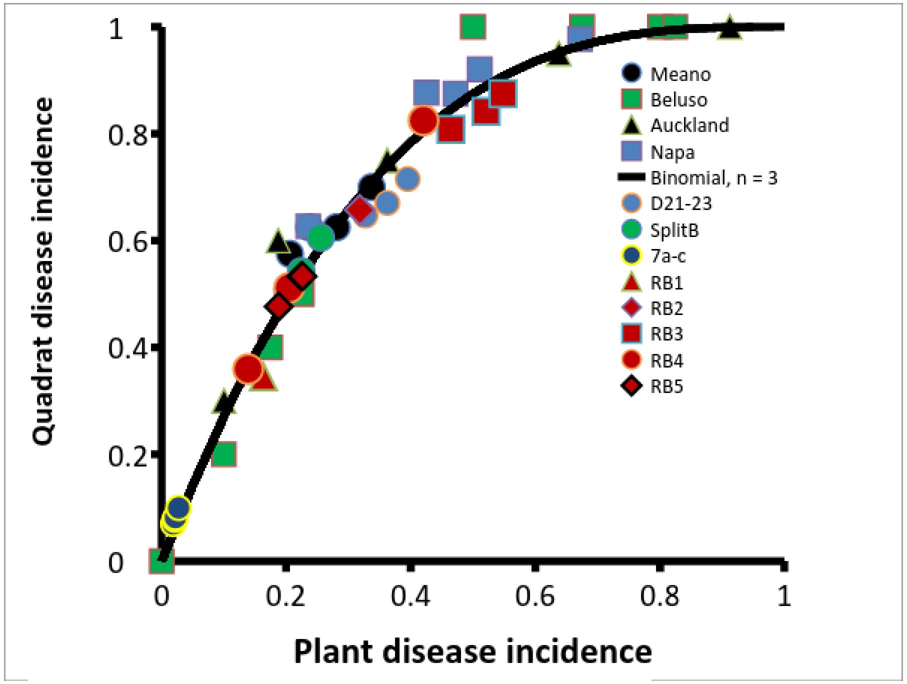
Examine the statistical properties of the incidence at two scales

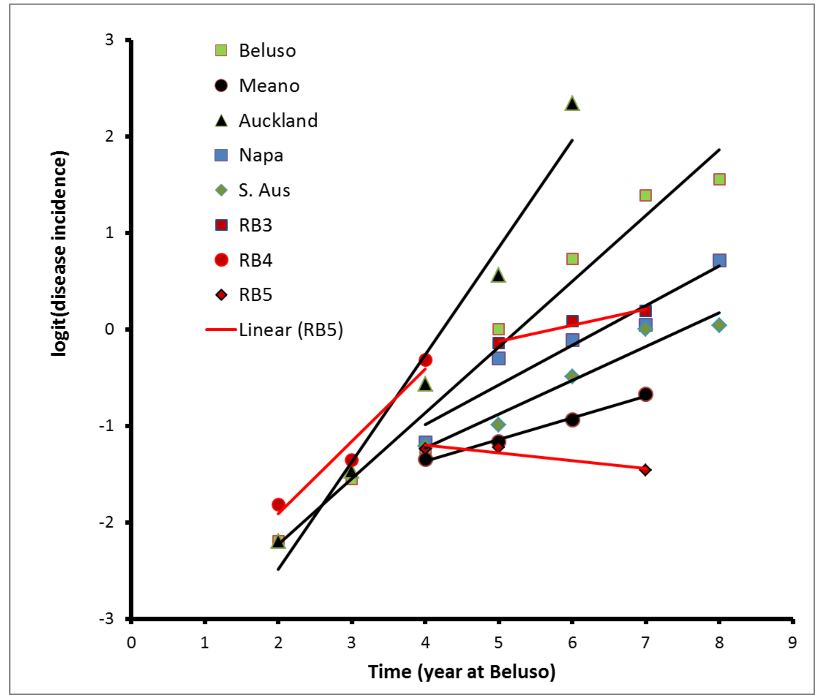
Where to sample?



Get as much coverage of different areas of a block as possible

Spatial pattern and rate of spread of Red Blotch and Leafroll compared





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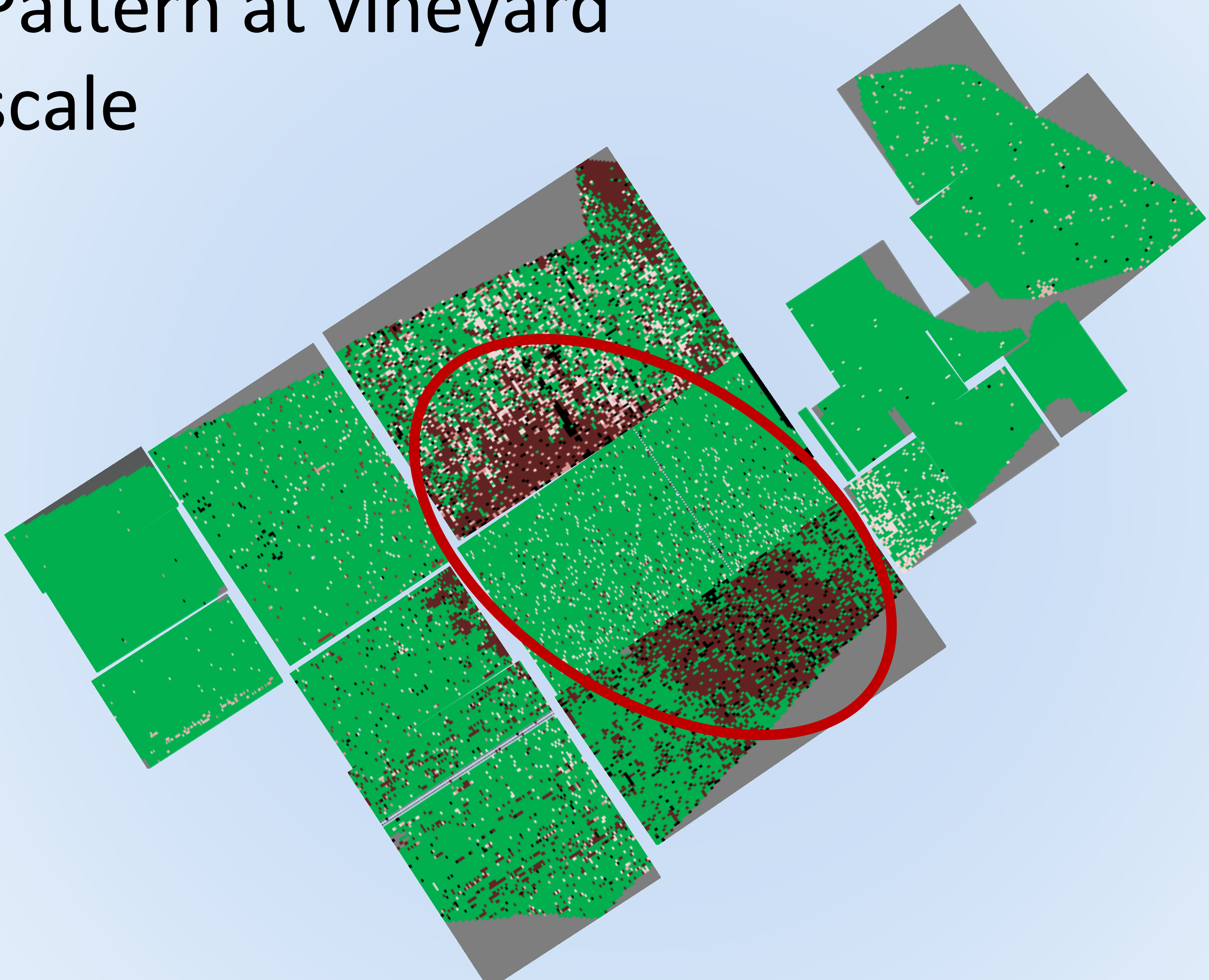
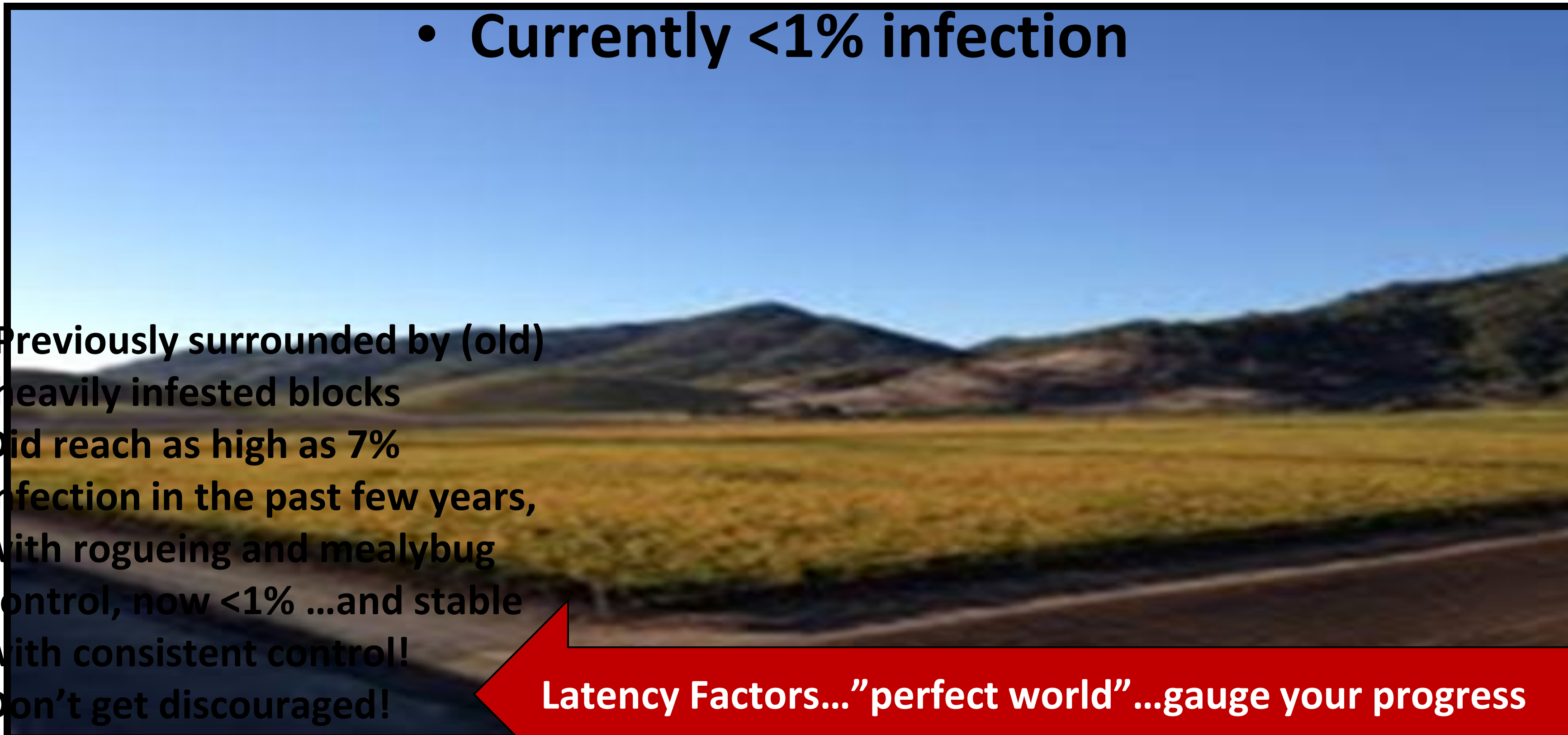
Pattern at vineyard scale



Effective leafroll control is possible

- Planted in 2002
- Currently <1% infection

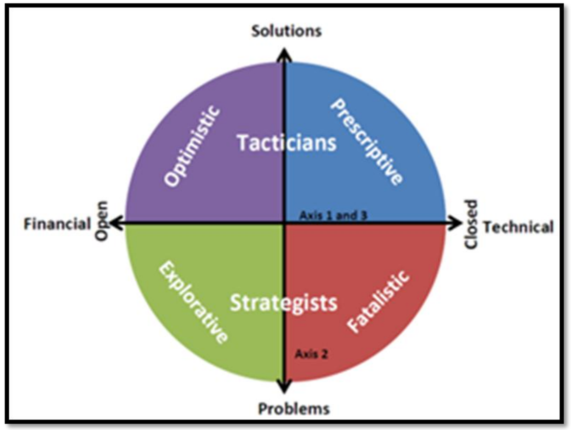


- Previously surrounded by (old) heavily infested blocks
- Did reach as high as 7% infection in the past few years, with rogueing and mealybug control, now <1% …and stable with consistent control!
- Don’t get discouraged!

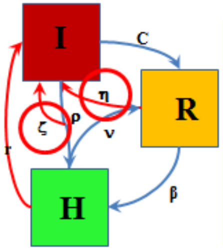
Latency Factors…”perfect world”…gauge your progress

Leafroll epidemiology

Sociological aspects

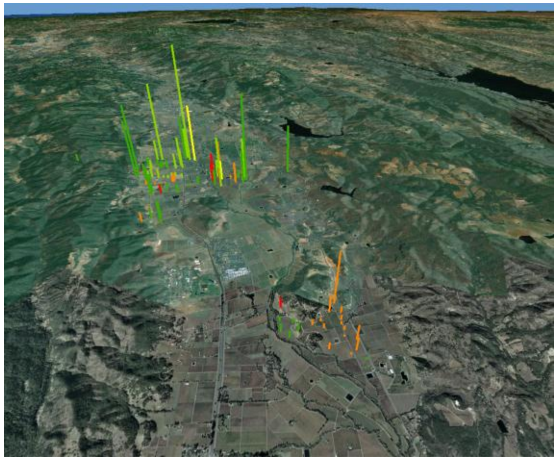


Inter-block meta-population model



$$\frac{dI}{dt} = rH + \eta R - cI - (1 - \zeta)\rho I$$
$$\frac{dH}{dt} = \beta R + (1 - \zeta)\rho I - rH - \nu H$$
$$\frac{dR}{dt} = cI + \nu H - \beta R - \eta R$$

LAMBA, Oakville



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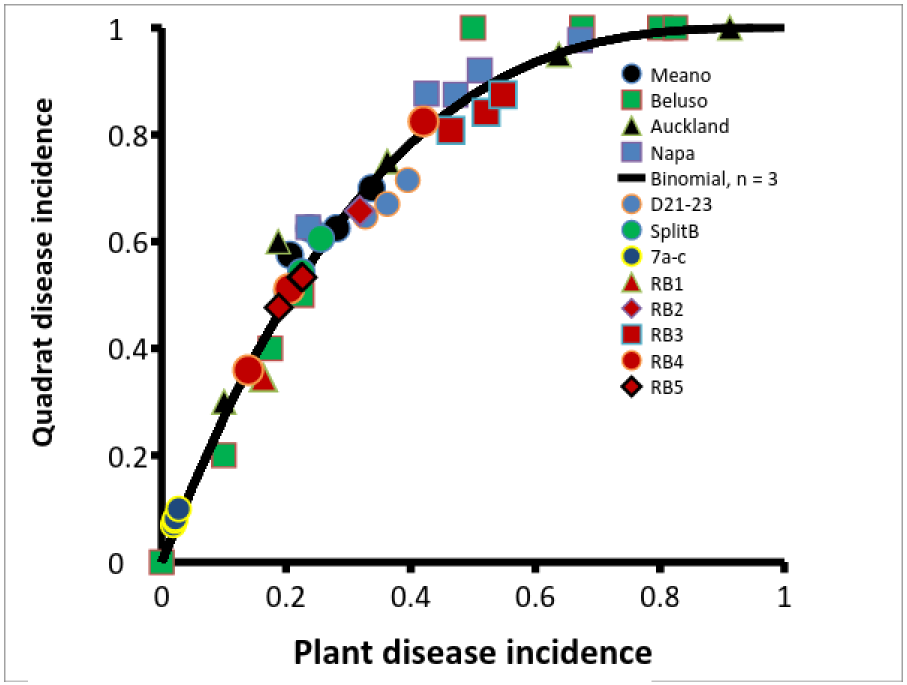
Locations of grower cooperation groups on virus management

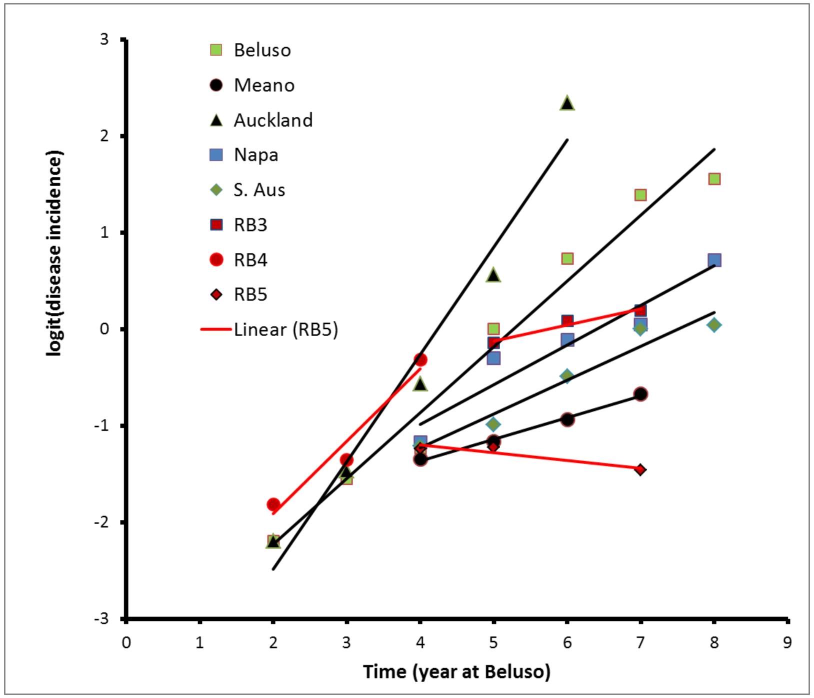
North Coast

Lodi?

Monterey

Spatial pattern and rate of spread of Red Blotch and Leafroll compared





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There are costs to making decisions of any kind. In varieties which express symptoms, does it always pay to take lab samples?

Visual rating	PCR-	PCR+
<=2	8	1
>2	1	8
	0.9	0.1
	0.1	0.9

Visual ratings against PCR results in 12 Zinfandel vines

Disease Ratings	PCR result
0	neg
0	neg
0	neg
1	neg
1	neg
1	neg
2	neg
2	neg
2	pos
3	pos
3	pos
3	pos
4	pos
4	pos
4	pos
5	pos
5	pos
5	neg

True Negative Proportion

True Positive Proportion

False Positive Proportion = 1-TNP

False Negative Proportion = 1-TPP

For decision-making under uncertainty, the likelihood ratios are important

LR+=	8
LR-=	0.125

Examples of prediction based on visual scoring

Positive prediction

Suppose Score>2

$p_{prior} = 0.36$, $odds_{prior} = 0.36/(1-0.36) = 0.53$

$odds_{post} = 8*0.53 = 4.24$

$p_{post} = 4.24/(1+4.24) = 0.81$

Negative prediction

Suppose Score<=T

$p_{prior} = 0.36$, $odds_{prior} = 0.36/(1-0.36) = 0.53$

$odds_{post} = 0.125*0.53 = 0.066$

$p_{post} = 0.066/(1+0.066) = 0.06$

Objective: The development of an ELISA assay for detection of GRBV

- The ELISA assay relies on the specific interaction between antibodies and a foreign protein, in this case the GRBV CP
- The antibodies must be specific to only the GRBV CP, and must reliably bind with it in all samples, even with low virus titer

- Benefits:
  - More cost effective than current d methods
  - Platform allows for high throughput testing
  - High degree of specificity and accuracy

What has been accomplished so far:

- The regions of the GRBV CP most likely to have high binding efficiency have been identified
- 7 monoclonal antibodies (MAbs) for these regions have been produced (the Mab production process takes ~ 4 months)
- Our lab has received these antibodies along with the antigenic peptides used to generate them (these are used as positive controls in our experiments)
- We are currently in the initial stages of testing the MAbs. This includes
  - Target protein (CP) size verification
  - Determining the specificity and binding capacity of each MAb in Western blots and ELISA
  - The MAbs are tested against both natured (from leaf tissue) and denatured (protein extraction in lab) GRBV CP

Basic pieces simplest case random pattern of infected plants sampling for incidence

- The central issue of concern is the plant disease incidence, *p*. This is the proportion of diseased plants in a batch.
- Any diagnostic test is characterized by its false positive rate, e_{fp} (1-specificty) and false negative rate, e_{fn} (1-sensitivity). Frequency of positive tests: $\bar{y} = p(1 - e_{fn}) + (1 - p)(1 - e_{fp})$
- It follows that an unbiased estimate of *p* is:
  - $p^* = \frac{\bar{y} - e_{fn}}{1 - e_{fn} - e_{fp}}$
- And sample size is: $N = \frac{\bar{y}(1-\bar{y})}{(1-e_{fn}-e_{fp})^2}\left(\frac{z_{\alpha/2}}{h}\right)$
- *h* is the half-width of the confidence interval around the mean – smaller *h* = more precision = higher sample size

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Sampling questions reveal shared responsibilities for quality

Example: Simple random sampling for incidence

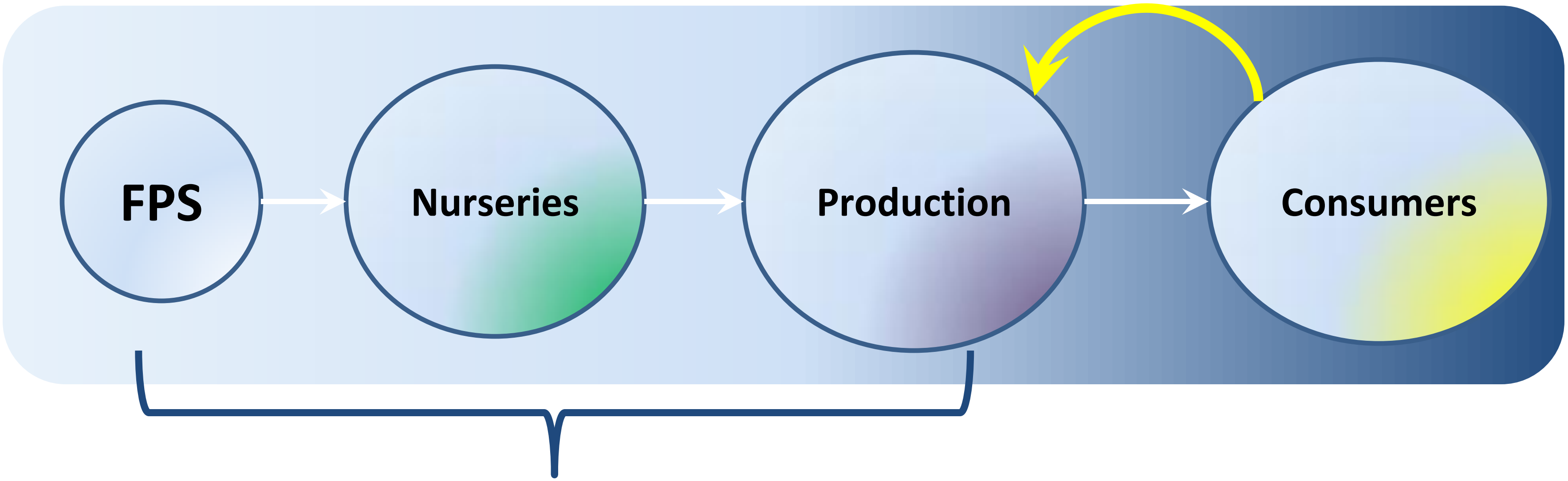
The anticipated disease incidence (nursery)

Sample size

$$N = \frac{\bar{y} \cdot (1 - \bar{y})}{(1 - \gamma - \eta)^2} \cdot \left(\frac{z_\alpha}{h} \right)^2$$

The desired level of confidence (nursery or grower)

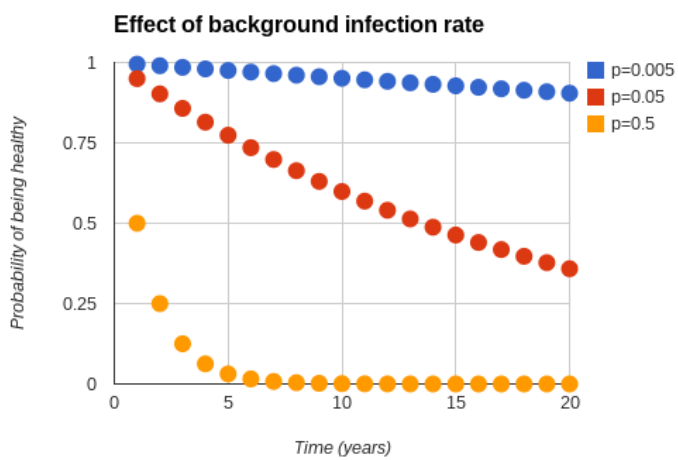
The diagnostic error rates (lab)



Sampling can be used to produce a supply chain with known performance for delivery of healthy vines

Assume background probability of vine being infected per year is p, so probability of staying healthy is $(1-p)$. Probability of being healthy after t years is $(1-p)^t$.

Let V_H = value of healthy vine
V_I = value of infected vine
Expected value of vine at time, t, = $\$[(1-p)^t V_H]+[1-(1-p)^t V_I]$



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Lifespan of vines depending on background infection rate and quality threshold