Transmission and spread of *Xanthomonas* in brassica transplants and implications for seed health

11th International Conference on Plant Pathogenic Bacteria
Edinburgh, Scotland, 10-14 July 2006

Steven Roberts
Plant Health Solutions – www.planthealth.co.uk

Outline

- Background thinking
- Transmission from seed to seedling
- Spread in transplants
- Implications for seed health

Black rot

- Caused by *Xanthomonas campestris* pv *campestris* (*Xcc*)
- V-shaped chlorotic, yellow lesions with blackened veins
- Systemic infection - stunted or dead plants
- Premature defoliation, secondary soft rots
- At least six races

Black rot - epidemiology

- *Xcc* well known as a seedborne pathogen
- Seeds are considered the primary source of inoculum and means of long-distance dissemination
- Crop debris and weeds *may* act as sources of infection but their relative importance not clear
- Insects may also spread the pathogen
- Control:
  - traditionally based on the use of disease-free (clean) seed
  - most commercial brassica seed is tested

Seed testing

- The problem with seed testing:
  - can never guarantee that a seed lot is completely healthy
- Can only test a sample:
  - tolerance std. = minimum % inf. seed which can be reliably detected (depends on sample size)
  - analytical sensitivity = minimum numbers of the pathogen which can be reliably detected (depends on assay design)

Seed testing

- What is 'clean' seed ?
  - *seed which has an infection level below the tolerance standard and analytical sensitivity of the seed test*

Design of Seed Health Assays

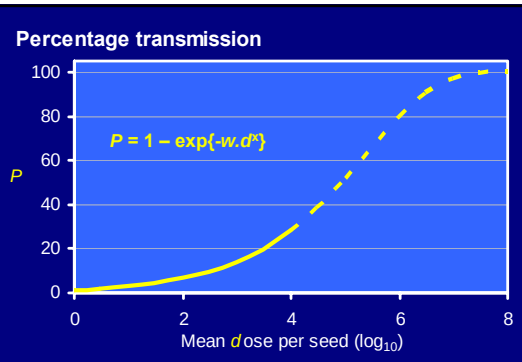
- Tolerance standards and analytical sensitivity should be defined which minimise disease risk and are based on an understanding of disease epidemiology
- The widely used tolerance standard of 0.01% is based on work done in USA on a direct-drilled crop (Schaad et al. 1990)
  - not appropriate for a transplanted crop
  - most vegetable brassica crops are transplanted

Drivers for the work

- In the field, symptoms appear suddenly with ~100% of plants affected
  - can this be explained low levels of seed infection and spread during plant raising ?
- Set effective seed health standards for current production practices
  - requires epidemiological models driven by:
    - transmission from seed to seedling
    - rate of spread during plant raising
    - rate of spread in field
    - relate to sensitivity/threshold of test method

Transmission from seed to seedling

- Seed inoculated with different doses of Xcc
- Sown in module '308' trays
- Grown on capillary matting (no overhead water) to avoid secondary spread
- Samples of plants collected and 'leaf washings' diluted and plated on selective media
- Proportion of plants contaminated was estimated by maximum likelihood
- 'One-hit' infection model fitted



P – probability of transmission *w* – 'one hit' probability (0.015)
d – number of *Xcc* per seed *x* – dose coefficient (0.034)

Probability of transmission for a single bacterium on a single seed is 0.015

Transmission from seed to seedling

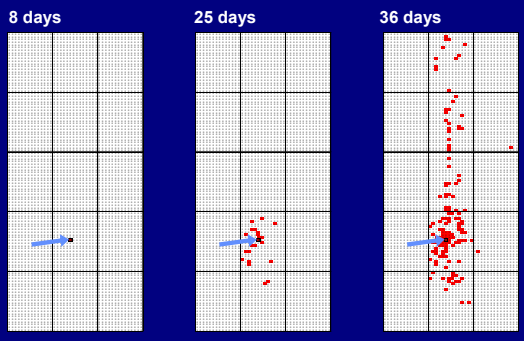
- More details in:
  - Roberts *et al.* (1999) *European Journal of Plant Pathology* 105, 879-889.

Rate of spread of in transplants

- Series of experiments
  - mimicking commercial production system with overhead gantry irrigation
- Single cell in block of 15 '308' trays sown with two inoculated seeds
- Symptoms 'mapped'
- Plants sampled and leaf washings done to detect *Xcc* on symptomless plants
- Models fitted to the data

Black rot - seedling symptoms

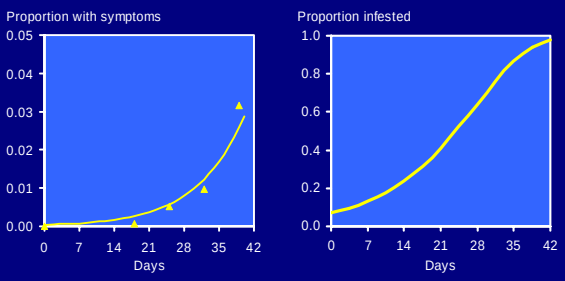
Spread in transplants



Symptoms, single primary infector, ~ 4,500 plants

Spread in transplants

Symptoms only half the story !



Spread in transplants

- Overhead gantry irrigation:
  - from one infested seed to nearly 4,500 contaminated seedlings in 6 weeks
  - final level 98%, limit of experiment

Spread in transplants

Model for overhead irrigation:

$$\ln\left(\frac{p_c}{1-p_c}\right) = \ln(a_c) + b_c \ln\left(c_c + \sqrt{k_c.x^2 + y^2}\right) + r_c.t$$

where:

p is the proportion of plants contaminated

x and *y* are the distance from primary infector,

t is the time, *b* is the gradient,

k is a directional scaling parameter

r is the rate, *a* and *c* are constants

Spread in transplants

- Range of parameter estimates obtained from different experiments

Experiment	Model parameters			
	k	$\ln(a_c)$	b_c	r_c
1 (1997)	15.9	4.76	-3.40	0.201
2 (1998)	15.9	-1.3	-1.90	0.342
3 (1999)	8.8	-0.77	-5.37	0.516

Spread in transplants

- Model parameters used to calculate the potential contamination in commercial-scale blocks of 100,000 transplants for different numbers of uniformly distributed primary infectors:
  - 1 primary ➔ 3 to 85%
  - 20 primaries ➔ 46 to 99%

Implications for seed health

- Now need to take account the probability of transmission occurring:
  - depends on the numbers of Xcc per infested seed:
    - 10 CFU ➔ 0.03
    - 1000 CFU ➔ 0.12
- Combining with potential contamination levels…..

Block of 100,000 transplants:

1 inf. seed in	% inf	CFU per inf seed	Prob. of transmission	Average % contam. of transplants
50,000	0.002	10	0.06	0 - 5
		100	0.12	1 - 11
		1000	0.23	1 - 21
25,000	0.004	10	0.14	1 - 13
		100	0.26	3 - 26
		1000	0.47	5 - 46
10,000	0.01	10	0.25	7 - 25
		100	0.46	12 - 45
		1000	0.72	19 - 71
5,000	0.02	10	0.44	20 - 44
		100	0.71	32 - 70
		1000	0.92	42 - 91

Implications for seed health

- Finally look at the probability of getting a positive seed test for the different initial % seed infestation and CFU per inf seed
- 'Standard' test method:
  - dilution plating on selective media
  - 3 sub-samples of 10,000 seeds in 100 ml
  - with centrifugation (~10x conc.) ➔ analytical sensitivity 1.5 CFU/ml
  - or no centrifugation ➔ 15 CFU/ml

Implications for seed health

- Probability of a positive test result, P_+ , depends on:
  - the probability of at least one infested seed being contained in the sample:

    $$P_{cont} = 1 - (1 - \theta)^n$$

    where θ is the true proportion of infested seeds, n is the sample size
  - if present, the probability of detecting an infested seed in a sub-sample:

    $$P_d = 1 - e^{-\lambda\nu}$$

    λ is the density of bacteria, ν is the effective volume plated
- Thus, $P_+ = P_{cont} \times P_d$

Definitions

- Unacceptable seed lot:
  - expected average contamination of transplants > 10% (arbitrary)
- Unacceptable seed test:
  - prob. of positive result << prob. of transmission for an unacceptable lot

Seed test results

1 inf. seed in	% inf	CFU per inf seed	Prob. of transmission	Average % contam. of transplants	Pr. + seed test	
					Cent.	No cent.
50,000	0.002	10	0.06	0 - 5	0.08	0.01
		100	0.12	1 - 11	0.39	0.08
		1000	0.23	1 - 21	0.45	0.39
25,000	0.004	10	0.14	1 - 13	0.13	0.01
		100	0.26	3 - 26	0.60	0.13
		1000	0.47	5 - 46	0.70	0.60
10,000	0.01	10	0.25	7 - 25	0.17	0.02
		100	0.46	12 - 45	0.82	0.17
		1000	0.72	19 - 71	0.95	0.82
5,000	0.02	10	0.44	20 - 44	0.33	0.04
		100	0.71	32 - 70	0.98	0.33
		1000	0.92	42 - 91	0.99	0.98

Implications for seed health

- Tolerance standard of 0.004% for transplanted crops ?
  - need to test 75,000 seeds for P ≥ 0.95
- Omitting centrifugation gives a greater risk of unacceptable tests
- Biggest risk of detection failures for epidemiological significant seed infestation:
  - low numbers of pathogen are spread over relatively larger numbers of infested seeds

Implications for seed health

- Seems counter intuitive:
  - tendency to assume that the biggest risk comes from seeds which have high level infestation
  - whilst true that they individually have a higher prob. of transmission, they are also easier to detect

Cautions

- Models, assumptions and calculations can be considered as imperfect, too simplistic:
- E.g.
  - seed tests assumed to be 'perfect' with no interfering saprophytes – in reality the prob. of detection will be lower
  - uniform distribution of primaries

Finally

- Need to consider both the analytical sensitivity and the tolerance standard (sample size) of the test when devising seed health tests for bacterial pathogens
- One simple way to improve sensitivity is to test the same total number of seeds in smaller sub-samples

The real workers !

- Josie Brough
- Paul Hunter
- Lea Hiltunen
- Barbara Everett
- Hort. Services staff at Warwick HRI