# BLACK ROT OF BRASSICAS (XANTHOMONAS CAMPESTRIS PV. **CAMPESTRIS**): SEED TRANSMISSION, SPREAD & STANDARDS

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## **Background**

- Xanthomonas campestris pv. campestris is well known as an important seed-borne pathogen of vegetable brassicas.
- Control by seed health testing can be very successful if effective seed health standards are applied.
- The problem is to define the standards and then design seed health assays to achieve those standards.
- For transplanted brassicas the final level of disease depends on:
  - the rate of transmission from seed to seedling
  - the rate of spread during plant raising
  - the rate of spread in the field
- Models have been developed for transmission and spread using experimental data and can be used to explore different scenarios.



### Models

- Transmission rate
  - Seed with different doses sown in glasshouse experiments.

$$P = 1 - \exp(-w.d^x)$$

Roberts et al (1999) European J. Plant Path. 105, 879-889.

- Spread in transplants
  - Series of glasshouse experiments with a single primary infector in block of ~4,500

 $\ln[p/(1-p)] = \ln(a) + b\ln[c + \sqrt{(k \cdot x^2 + y^2)}] + r.t$ Roberts et al (2006) Plant Path. 56, 391-401.

- Spread in the field
  - Series of field experiments with a single infector in block of ~1,000 transplants

$$\ln[p/(1-p)] = \ln(a) + b\ln[c+s] + r.t$$

- Seed testing
  - Dilution plating, probability of detection:

$$F(\theta, m, p_s) = \sum_{i=1}^{m} bi(j, m, \theta) \times [1 - (1 - p_s)^j]$$





First symptoms on cotyledons are difficult to spot, and pathogen spread will have already occured.

# **Example risk scenarios**



- Block of 100,000 transplants
- Seed infestation from 0.002 to 0.02%
- 10<sup>1</sup> to 10<sup>3</sup> CFU per infested seed
- Target overall risk of 10%

Seed inf.		Prob	Spread <sup>3</sup>	Pr +ve test <sup>4</sup>		Overall risk <sup>5</sup>	
%	CFU <sup>1</sup>	Trans. <sup>2</sup>	Avg %	1 x 10k	6 x 10k	1 x 10k	6 x 10k
0.002	10 <sup>1</sup>	0.06	0-5	0.04	0.20	0.05	0.05
	$10^{2}$	0.12	1-11	0.16	0.65	0.10	0.04
	$10^{3}$	0.23	1-21	0.18	0.70	0.18	0.07
0.004	10 <sup>1</sup>	0.11	1-11	0.07	0.35	0.10	0.07
	10 <sup>2</sup>	0.22	2-21	0.29	0.87	0.15	0.03
	10 <sup>3</sup>	0.40	4-39	0.33	0.91	0.27	0.04
0.010	$10^{1}$	0.25	7-25	0.17	0.66	0.21	0.09
	10 <sup>2</sup>	0.46	12-45	0.58	0.99	0.19	0.00
	10 <sup>3</sup>	0.72	19-71	0.63	1.00	0.27	0.00
0.020	10 <sup>1</sup>	0.44	20-44	0.30	0.89	0.31	0.05
	10 <sup>2</sup>	0.71	32-70	0.82	1.00	0.13	0.00
	<b>10</b> <sup>3</sup>	0.92	42-91	0.86	1.00	0.12	0.00

<sup>3</sup> No. of bacteria per infested seed. <sup>2</sup> Probability of transmission. <sup>3</sup> Predicted disease incidence 6 weeks after sowing. <sup>4</sup> Prob. of positive test result for a test on one or six sub-samples of 10,000 seeds, with a centrifugation step. <sup>5</sup> Probability of transmission x probability of a negative test result; values in red are considered to represent an unacceptable level of risk (i.e. > 10%).

### Conclusions

- Effective control of X. c. pv. campestris in transplanted brassicas can be achieved by testing  $6 \times 10,000$  seeds with centrifugation.
- Seed lots with a relatively high proportion of infested seeds but low numbers of bacteria per seed represent the greatest risk of detection failures.

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# Black rot of brassicas (Xanthomonas campestris pv. campestris): seed transmission, spread and standards

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Xanthomonas campestris pv. campestris is well known as an important seed-borne pathogen of brassicas. Seed health assays should be designed to have a high probability of detecting unacceptable seed lots; there has been much discussion over the years recent of the value of the most sensitive detection assays and the tolerance standards required to achieve satisfactory control. Mathematical models have been developed to describe transmission of the pathogen from seed to seedling, subsequent spread in module-raised brassica transplants, and spread in the field. The transmission model relates the probability of transmission to the mean dose of bacteria per seed and the spread model relates the proportion of plants contaminated to the distance from the primary infector. Using these models, with different initial parameters, the potential for development of disease epidemics can be explored for negative results obtained by seed health assays with different sensitivities (detection limits) and tolerance standards. Examples of different scenarios will be presented, and suggest that the greatest risk arises when negative test results are obtained from seed lots with a relatively high proportion of infested seeds but low number of bacteria per seed.

### **Transmission model**

$$P = 1 - \exp(-w.d^x)$$

where P is the probability of transmission, w is the one-hit probability, d is the mean dose per seed, x is the dose coefficient.

### Spread in transplants model

$$\ln[p/(1-p)] = \ln(a) + b\ln[c + \sqrt{(k \cdot x^2 + y^2)}] + r \cdot t$$

where p is proportion of plants infected or contaminated, a is the intercept, b is the gradient, c is a truncation parameter, k is a directional scaling parameter, x and y are distances from the primary infector, r is the relative infection rate, t is time

### Spread in the field model

$$\ln[p/(1-p)] = \ln(a) + b\ln[c+s] + r.t$$

where p is proportion of plants infected or contaminated, a is the intercept, b is the gradient, c is a truncation parameter, k is a directional scaling parameter, s is the distance from the primary infector, r is the relative infection rate, t is time

### Seed testing model:

$$p_* = F\left(\theta, m, p_s\right) = \sum_{j=1}^m bi(j, m, \theta) \times \left(1 - (1 - p_s)^j\right)$$

where  $p_+$  is the probability of a positive test result,  $\theta$  is the proportion of seeds infested, m is the sub-sample size, bi() is an individual term in the binomial expansion,  $p_s$  is the test sensitivity.

$$p_s = 1 - \exp(-vn/V)$$

where n is the CFU per seed, v is the volume plated, V is the extraction volume.