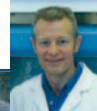


TRANSMISSION AND SPREAD OF XANTHOMONAS CAMPESTRIS PV CAMPESTRIS IN BRASSICA TRANSPLANTS: IMPLICATIONS FOR SEED HEALTH STANDARDS

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Background

- *Xanthomonas campestris* pv. *campestris* (Xcc) 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 dispute in recent years over the value of the most sensitive detection assays and the tolerance standards required to achieve satisfactory control.
- Mathematical models have been developed for transmission of the pathogen from seed to seedling and for subsequent spread in module-raised brassica transplants.
- Using these models, with different initial parameters, allows exploration of the potential for development of disease epidemics for negative results obtained by seed health assays with different sensitivities (detection limits) and tolerance standards.

Transmission model

Transmission from seed to seedling:

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

where:

- P - probability of transmission
- w - 'one-hit' probability (0.014)
- d - dose (number of Xcc per seed)
- x - dose coefficient (0.32)

Spread model

Spread in transplants:

$$\ln[p_c / (1 - p_c)] = \ln(a_c) + b_c \ln[c_c + (k \cdot x^2 + y^2)^{1/2}] + r_c \cdot t$$

where:

- p_c - proportion of plants contaminated
- a_c - intercept
- b_c - gradient
- c_c - truncation parameter
- k - directional scaling parameter
- x, y - distance from primary infector in x and y directions
- r - relative contamination rate
- t - time

Example scenario

- Batch of 100,000 transplants with overhead gantry irrigation.
- Seed infestation levels: 0.02 to 0.002%.
- Xcc: 10 to 1000 cfu per infested seed.
- Range for % contaminated transplants results from different initial model parameters.
- Seed test: 3 x 10,000 seeds with or without centrifugation. Probabilities based on theoretical detection limits.

Infestation level	1 seed in		cfu per inf seed	Prob. of transmission	Average % contam. transplants	Seed test	
	1 seed in	%				Prob. +ve (cent.)	Prob. +ve (no cent.)
50,000	0.002		10	0.03	0 - 3	0.08	0.01
			100	0.06	0 - 6	0.39	0.08
			1000	0.12	1 - 11	0.45	0.39
25,000	0.004		10	0.14	2 - 13	0.13	0.01
			100	0.26	3 - 26	0.60	0.13
			1000	0.47	6 - 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	1.00	0.98

- Unacceptable lots - expected contamination of transplants > 10% (conservative estimate) ?
- Unacceptable test - prob. of positive result << prob of transmission for unacceptable lot ?
- Omitting centrifugation gives a greater risk of unacceptable tests.
- Greatest danger of detection failures is from seedlots with high % infestation but low cfu per seed.

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MODEL PARAMETERS AND DETAILS OF CALCULATIONS

Spread model

Model parameters were obtained from three different experiments in which spread of *Xcc* was monitored for up to six weeks in blocks of module brassica transplants with a single primary infector (cell sown with inoculated seed(s)).

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

Using these model parameters the expected proportions of contaminated transplants were calculated for a block of approximately 100,000 transplants, assuming uniform distribution of infested seedlings and assuming 100% transmission. The average % contamination of transplants in the table was calculated by multiplying the expected proportion obtained from the spread models above by the probability of transmission.

Probability of positive seed tests

For the purposes of the calculations the following assumptions were made about the seed tests:

- three sub-samples of 10,000 seeds were tested (i.e. total number = 30,000);
- each sub-sample of 10,000 seeds was suspended in 100 ml of saline;
- two plates were used for each dilution plated;
- there was no interference from background saprophytes.

The probability of a positive test result, P_+ , depends on:

- (a) 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 in the lot and n is the total number of seeds in the sample.

- (b) if present, the probability of detecting an infested seed in a sub-sample:

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

where λ is the mean density of bacteria in the suspension (i.e. the number of bacteria per infested seed divided by the volume in which the sub-sample is suspended) and v is the effective volume plated (2 ml for centrifugation and 0.2 ml without centrifugation).

$$\text{i.e. } P_+ = P_{cont} \times P_d$$

Selected References

- Roberts, S.J., Phelps, K., Taylor, J.D. and Ridout, M.S. (1993) Design and interpretation of seed health assays. In: Sheppard, J.W., (Ed.) *Proceedings of the First ISTA Plant Disease Committee Symposium on Seed Health Testing, Ottawa, Canada*, pp. 115-125. Ottawa, Canada: Agriculture Canada. [http://www.planthealth.co.uk/downloads/Design_and_interp.pdf]
- Roberts, S.J., Hiltunen, L.H., Hunter, P.J. and Brough, J. (1999) Transmission from seed to seedling and secondary spread of *Xanthomonas campestris* pv. *campestris* in Brassica transplants: effects of dose and watering regime. *European Journal of Plant Pathology* **105**, 879-889.
- Roberts, S.J. (1999) Thresholds, standards, tests, transmission and risks. In: Sheppard, J.W., (Ed.) *Proceedings of 3rd ISTA Seed Health Symposium, Ames, Iowa, USA, 16-19 August 1999.*, pp. 20-24. Zurich, Switzerland: ISTA. [http://www.planthealth.co.uk/downloads/Thresholds_and_standards.pdf]
- Roberts, S.J., Brough, J., Everett, B. and Redstone, S. (2004) Extraction methods for *Xanthomonas campestris* pv. *campestris* from brassica seed. *Seed Science and Technology* **32**, 439-453.