Transmission and spread of *Xanthomonas campestris* pv. *campestris* in brassica transplants: implications for seed health standards

S J Roberts
Plant Health Solutions, 20 Beauchamp Road, Warwick, CV34 5NU, UK
Email: s.roberts@planthealth.co.uk

Summary

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. Mathematical models have been developed both for transmission of the pathogen from seed to seedling and subsequent spread in module-raised brassica transplants. 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.

Introduction

Xanthomonas campestris pv. *campestris* (*Xcc*) is well known as an important seedborne pathogen of brassicas. Seed health assays should be designed to have a high probability of detecting unacceptable seedlots. The problem is to define an 'unacceptable seedlot', and in recent years, there has been much dispute over the value of the most sensitive seed health assays and the tolerance standards required to achieve satisfactory control of *Xcc* in brassicas. Schaad *et al.* (1990) suggested a tolerance standard of 0.01% for a direct-drilled brassica crop, but that this was inadequate for transplant production. Most vegetable brassicas are grown as transplanted crops, but nevertheless most seed is still tested to a tolerance standard of 0.01%. This paper will present the results of work done over several years to develop models to describe the transmission and spread of *Xcc* which have then allowed us to examine the potential development of disease epidemics for seedlots with different seed health scenarios and the likelihood of their detection in seed health assays with different sensitivities.

Models

A model for the transmission of *Xcc* from seed to seedling was devised using data from glasshouse experiments. Seed was inoculated with different concentrations of bacteria, and sown in commercial module trays and subjected to different watering regimes (Roberts *et al.*, 1999). Visible symptoms were recorded, and leaf washings were carried out to detect the pathogen on symptomless plants. The results were consistent with a one-hit model for infection/transmission:

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

where P is the probability of transmission, w is the 'one-hit' probability, d is the dose (number of *Xcc* per seed) and x is a dose coefficient.

To continue to a second page, once the last line of text fills the box above you must move to the next page

A model for the spread of *Xcc* in brassica transplants was developed using data from a series of glasshouse experiments designed to simulate a typical commercial module plant raising system with overhead gantry irrigation (Roberts *et al.*, 2006). Primary inoculum was introduced as inoculated seeds in one or more cells. Disease symptoms were mapped and the presence of the pathogen on samples of plants was monitored by leaf washing, dilution and plating on a selective medium. Spread of symptoms and spread of contamination followed a similar pattern, but the proportion of plants contaminated was much greater than the proportion showing symptoms; approaching 100% after six weeks in the gantry-watered trays within 50 plants distance from a single primary infector. Models relating the proportion of plants with symptoms, or contaminated, to the distance from primary infector and time since sowing were fitted to the data:

$$\ln[p/(1 - p)] = \ln(a) + b\ln[c + (k.x^2 + y^2)^{½}] + r.t$$

where p is the proportion of plants contaminated, a is an intercept parameter, b is the gradient, c is a truncation parameter, k is a directional scaling parameter, x, y are the distance from the primary infector in the x and y directions, r is the relative contamination rate, and t is time.

These models were used to explore the potential for development of disease epidemics in commercial-scale blocks of transplants for seedlots with different proportions of seed infested and different numbers of bacteria on those infested seeds. Using model parameters from different spread experiments, 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 was then calculated by multiplying the expected proportion obtained from the spread models above by the probability of transmission obtained from the transmission model for the different seed infestation scenarios.

For each seed infestation scenario, the probability of detection was also calculated for seed health assays with different sensitivities (detection limits; resulting from the inclusion/omission of a centrifugation step). The probability of at least one infested seed being contained in the sample is given by:

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

where θ is the true proportion of infested seeds in the lot and n is the total number of seeds in the sample. Then, if present, the probability of detecting an infested seed in a sub-sample is given by:

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

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. Thus the probability of a positive result for the test is given by:

$$P_+ = P_{cont} \times P_d$$

Arbitrarily, an unacceptable seedlot was defined as one in which the expected average contamination of transplants was greater than 10% at the time of planting (six weeks after sowing) and an unacceptable test was indicated when the probability of detection was less than the probability of transmission for an unacceptable lot.

Results and Conclusions

Table 1. Example scenarios for different proportions of infested seed, and numbers of *Xanthomonas campestris* pv *campestris* per infested seed, together with the probability of a positive test result with (Cent) and without (No cent) centrifugation to improve analytical test sensitivity.

1 inf. seed in:	% infested	CFU per inf. seed	Prob. of transmission	Average % contam. of transplants	Prob. +ve 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[2]
		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

[1] unacceptable tests

Some example scenarios are shown in Table 1; starting with seed infestation levels ranging from 1 in 5,000 to 1 in 50,000 seeds and mean numbers of *Xcc* per infested seed from 10 to 1000. The remaining columns show the results of running the transmission and spread models, together with the probabilities of obtaining a positve seed test result with and without a centriguation step.

The transmission and spread models suggested that the high levels of disease incidence often seen in the field can be explained by rapid rates of pathogen spread during plant-raising and that the widely used tolerance standard for seed health testing (0.01%) is inadequate and should be revised to 0.004%. Given the potential difficulty of achieving this standard (it requires 75,000 seeds to be tested), in addition to seed health testing, control should focus on raising transplants under conditions that minimise the rate of disease/pathogen spread.

The results also indicated that omitting the centrifugation step (as in the current ISTA method) gives a greater risk of unacceptable tests. The greatest danger of detection failures occurs with seedlots with a relatively high percentage infestation but low numbers of bacteria per seed, and highlights the importance of both the detection limits and analytical sensitivity when designing effective seed health assays.

Acknowledgements

This work was done at Horticulture Research International, Wellesbourne and was funded by the UK Department for the Environment, Food and Rural Affairs.

Selected References

Roberts SJ; Phelps K; Taylor JD; Ridout MS (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 SJ; Hiltunen LH; Hunter PJ;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 SJ (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 SJ; Brough J; Everett B; Redstone S (2004). Extraction methods for *Xanthomonas campestris* pv. *campestris* from brassica seed. *Seed Science and Technology* **32**, 439-453.

Roberts SJ; Brough J; Hunter PJ (2007). Modelling the spread of *Xanthomonas campestris* pv. *campestris* in module-raised brassica transplants. *Plant Pathology* **56**, 391–401.

Schaad NW; Sitterly WR; Humaydan H (1990). Relationship between incidence of seedborne *Xanthomonas campestris* pv. *campestris* to black rot of crucifers. *Plant Disease*, **64**, 91-92.