European Journal of Plant Pathology **105:** 879–889, 1999.

Transmission from seed to seedling and secondary spread of *Xanthomonas campestris* pv. *campestris* in Brassica transplants: effects of dose and watering regime

S.J. Roberts, L.H. Hiltunen, P.J. Hunter and J. Brough
Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK (Fax: +44 1789 470552; E-mail: steve.roberts@hri.ac.uk)

Accepted 1 October 1999

Key words: black rot, epidemiology, modelling, seed pathology

Abstract

The effects of inoculum load and watering regime on the transmission of *Xanthomonas campestris* pv. *campestris* from seed to seedlings of cauliflower were investigated. Seed, inoculated with different concentrations of bacteria, was sown in commercial module trays and subjected to four different watering regimes: high frequency overhead spray, low frequency overhead spray, high frequency capillary and low frequency capillary. Visible symptoms were recorded and leaf washings were carried out to detect the pathogen on symptomless plants. The effects of treatments on symptoms and on the proportion of contaminated but symptomless plants was similar. Initially, they were influenced only by the dose of bacteria with little difference between the watering regimes, but later the proportion of plants with symptoms was greater for plants subjected to overhead watering, due to spread and secondary infection. Generalised linear models were fitted to the data relating the proportion of symptomless contaminated plants or the proportion of plants with symptoms, p, to the mean dose of bacteria per seed, d, and the number of overhead waterings, *noh*. The equations were: $p = 1 - \exp(-0.014 \cdot d^{0.32} \cdot noh^{0.045})$ for symptomless contaminated/infected plants and $p = 1 - \exp(-0.0056 \cdot d^{0.44} \cdot noh^{0.014})$ for plants with symptoms. These models indicated that the one-hit probability for transmission of the pathogen (i.e. with/without visible symptoms) was 0.014 and for infection (i.e. with visible symptoms) was 0.0056.

Introduction

Black rot of crucifers is a primarily seedborne (Cook et al., 1952; Schaad et al., 1990) disease caused by the bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*). It is one of the most important diseases of brassicas worldwide (Williams, 1980) and besides causing increasing problems to UK growers over recent years, has also been identified as a priority disease by the International Seed Health Initiative.

Schaad et al. (1990) suggested that a tolerance standard for seed infection of 0.01% was adequate for control of the disease in direct seeded crops but that a zero tolerance was necessary for transplant production. Most vegetable brassica crops in the UK and Europe are raised from transplants, but most commercial brassica seed is usually tested for the presence of *Xcc* only to the 0.01% tolerance standard. It is therefore feasible that one reason for recent increases in disease is the trend to use module-raised transplants supplied by a small number of specialist plant-raising nurseries where the 0.01% tolerance standard may be inadequate. However, there is little quantitative data on the transmission of bacterial pathogens from contaminated/infected seeds to seedlings or on the factors which affect this transmission. Some work has been done on the transmission of pea blight (*Pseudomonas syringae* pv. *pisi*) from seed to seedling (Roberts et al., 1996) where the major

effects of inoculum load and soil moisture on disease incidence in emerged seedlings were demonstrated and quantified.

The aim of the study reported here was examine the effects of inoculum dose and watering regime on the transmission of *Xcc* from cauliflower seeds to seedlings in a module plant-raising system.

Material and methods

Experimental design and layout

Artificially infested seeds with four levels of inoculum (A, high, 3.5×10^3 cfu seed^{-1}; B, medium, 1.5×10^2 cfu seed^{-1}; C, low, 1.4 cfu seed^{-1}; D, uninoculated control) were sown in commercially used '308' module trays (609 × 400 × 45 mm, 22 × 14 cells, 13.5 cm^3 cell volume, Linpac Material Handling, Dunstable, UK); all seeds in each tray were inoculated. Trays were subjected to four different watering regimes (high frequency spray, HS; low frequency spray, LS; high frequency capillary, HC; low frequency capillary, LC; see Table 1). A systematic experimental design was used in order to facilitate watering, and minimise the likelihood of interference between treatments and also because positional effects in the glasshouse (volume ~ 500 m^3) were considered to be minimal. Trays were set out in eight blocks of four on each of two benches in a glasshouse with a set temperature of 18 °C and venting at 20 °C. Each block of four trays represented the same inoculum dose and were set out in order of inoculum dose. One bench was used for the high frequency watering treatments and the other for the low frequency watering treatments, with half of each bench allocated to either spray or capillary watering. Perspex screens were placed between the spray and capillary watering regimes to prevent spray drift. Each block of four trays was separated from the next by a gap of 0.4 m (width of one tray) and the two benches were separated by an empty bench (1.5 m) (Figure 1). The experiment was run for 6 weeks, the duration of plant raising in normal commercial practice.

Seed and inoculation

Untreated cauliflower seed of a winter cultivar known to be susceptible to black rot (cv. Miracle) was obtained

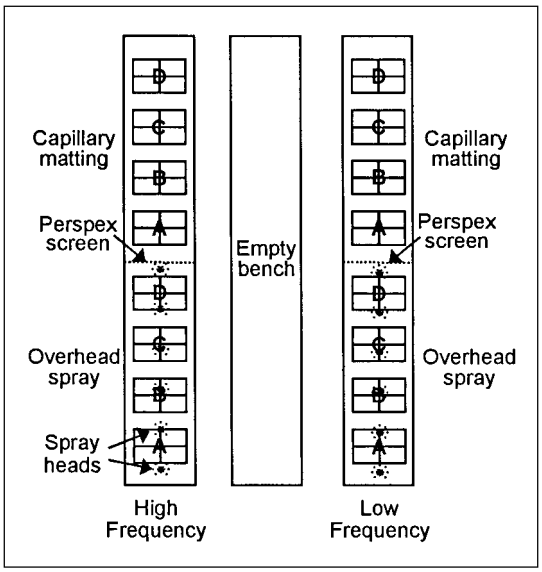

Figure 1. Layout of trays and treatments on glasshouse benches.

Table 1. Watering regimes

Code	Description	Duration (min)	Times of application	Amount per day	Mean compost moisture to 95% emergence	
					(% d.w.)	($\times 10^{-3}$ mPa)
HS	High frequency overhead spray	2	0600, 1100, 1600 every day	2.5 ml cell^{-1} 4.4 mm	472	−0.75
LS	Low frequency overhead spray	2	0600 alternate days[1]	0.4 ml cell^{-1} 0.7 mm	392	−1.4
HC	High frequency capillary matting	2	0600, 1100, 1600 every day	—	506	−0.60
LC	Low frequency capillary matting	2	0600 alternate days[1]	—	419	−1.1

[1]Plus minimal additional watering to maintain plants.

from Elsoms Seeds, Spalding, Lincolnshire, UK. An isolate of *Xcc* (3818A) was recovered from storage on glass beads at −76 °C onto YDC agar (g l^{-1} : yeast extract, 10; $CaCO_3$, 20; Bacto agar, 15; glucose 20). This isolate had been obtained from a crop of cauliflower cv. Miracle which was part of a field trial in Cornwall, UK. After 24 h at 30 °C, bacterial growth was scraped from the plate with a sterile spatula and suspended in 10 ml of sterile saline (0.85% NaCl) to give a concentration of approx. 10^9 cfu ml^{-1}. This was diluted with 40 ml of saline to provide the highest inoculum concentration (A) and two serial 50-fold dilutions were prepared from this suspension to provide lower inoculum concentrations (B and C). Aliquots of seed (25 g, ∼ 6000 seeds) were immersed in the bacterial suspensions (A, B and C) and a vacuum applied for 5 min. The vacuum was released and the seed drained. It was then spread thinly in shallow trays on paper towels and left to dry overnight at room temperature in the airflow of a fume hood. Seeds for the uninoculated control (D) were immersed in sterile saline and then treated as described above. Bacterial numbers in the inocula were estimated by dilution and plating onto YDC using the drop method of Miles and Misra (1933). After drying, the seeds were stored in sealed polythene bags at ∼ 4 °C until sowing 7 days later.

Seed testing

The number of bacteria on the inoculated seed was determined 2 and 9 days after inoculation (i.e. before and after sowing) following a method based on International Seed Testing Association Working Sheet No 50. For each inoculum concentration, one sample of 30 seeds, three samples of ten seeds and six samples of one seed were soaked for 2.5 h at room temperature in 2, 1 and 1 ml of sterile saline, respectively. Three samples of 1000 uninoculated seeds were soaked in 10 ml of sterile saline. In the case of the 1000, 30 and 10 seed samples, the resulting suspensions were diluted and 100 µl of each dilution spread on plates of FS agar medium (Schaad, 1989) with a bent glass rod. In the case of the single seed samples 4 × 100 µl of the undiluted suspensions were spread onto plates of FS agar. Plates were incubated at 30 °C for 3 days before counting the number of typical *Xcc* colonies. The identity of a selection of typical colonies was confirmed using *Staphylococcus aureus* slide agglutination (Lyons and Taylor, 1990) with an antiserum specific for *Xcc*.

Tray filling and sowing

Module '308' trays were filled loosely with Levington F1 compost. The surface was levelled and firmed so that the surface of the compost was 0.5–1 cm below the top of the tray. The seeds were sown with an automatic sower (Hamilton Seeder), one seed per module, and the trays covered with the same compost up to the top of the tray. To obviate the effect of any cross-contamination, seeds were sown sequentially with uninoculated seeds first and those receiving the highest dose last. Trays were numbered in order of sowing and transferred to the glasshouse and *all* trays were watered using the overhead sprays set up for the spraying treatments. The trays in the high frequency regimes were watered for 14 min, to bring the water content of the compost up to the maximum i.e. field capacity, and the trays in the low frequency regimes were watered for 7.5 min. (to bring the water content up to 50% of the maximum).

Watering regimes

The four different watering regimes are summarised in Table 1. For the capillary matting treatments, benches were covered with a layer of polythene sheeting, then capillary matting, then perforated black polythene. Water was applied via seep hoses, which were arranged across the bench at 0.4 m intervals, i.e. one seep hose on each side of every tray. For the overhead spraying treatments, Eintal spray nozzles (Access Irrigation Limited, Northampton, UK) were installed on risers 0.3 m high and 0.9 m apart to provide an overlapping spray pattern. The volume of water delivered by the spray heads was estimated by collecting water in ten 16.5 cm diameter dishes spaced out on the benches.

Plants in the high frequency watering regimes (both overhead spray and capillary matting) were watered daily at 0600, 1100 and 1600 h for 2 min on each occasion. Plants in the low frequency regimes were watered on alternate days at 0600 h for 2 min on each occasion. Watering was controlled by an electronic irrigation controller connected to solenoid valves. Plants in the low frequency watering treatments were also given additional water by manually switching the irrigation controller when necessary during warmer weather to prevent wilting.

From 16 days after sowing, a liquid feed (0.33 g l^{-1} KNO_3; 40 ppm N) was applied to all treatments, with every watering, by dosing the water supply line with a concentrated stock solution.

Records

Compost moisture. The moisture content of the compost was determined twice weekly by removing individual cells and determining wet and dry weights. One cell was randomly sampled for each block of four trays, i.e. four samples per watering regime. Samples were weighed in aluminium foil dishes, dried in an oven (Hotbox Oven, Gallenkamp, UK) at 150 °C for 2 h and then reweighed.

Temperature. Air temperature was recorded at hourly intervals by the glasshouse environmental computer.

Emergence. The number of emerged seedlings was counted at 6, 8, 10 and 13 days after sowing. The counts were made in one tray chosen at random from each block of four trays.

Disease symptoms. The number of plants showing black rot symptoms (chlorosis and/or necrosis with blackened veins) was recorded in one tray in each treatment at 23 days after sowing. A complete record of the number of plants with symptoms in every tray was recorded at 29–30 days after sowing. Numbers of emerged seedlings and empty/sampled cells were also recorded for each row of 22 cells in each tray, at each recording.

Isolations. In order to confirm that the observed black rot symptoms were caused by *Xcc*, isolations were attempted from at least one plant from each (inoculated) treatment with symptoms considered to be typical. Small (1–2 mm^2) pieces of diseased tissue were comminuted in a drop sterile saline on a sterile microscope slide and observed by phase contrast light microscopy before streaking out the suspensions on plates of YDC. The identity of isolates was confirmed by slide agglutination.

Leaf washings. Leaf washings to estimate the proportion of contaminated/infected symptomless plants were done at two and six weeks after sowing. At the first sampling, three samples of 64 plants, one sample of 19 plants and four samples of 6 plants were randomly selected from two trays in each treatment, i.e. eight samples from each treatment. The sample sizes were chosen using a Fortran program to obtain the maximum likelihood estimate of the proportion infected using a single stage design and based on a prior range of 0.5–50% (Ridout, 1995). At the second sampling, samples were collected from the two trays which had not been sampled at the first sampling and sample sizes varied for different treatments to take into account the results of the first sampling.

Plants were cut off just below the cotyledons using sterile scissors and put into clean polythene bags. A separate polythene bag was used for each sample of plants and scissors were disinfected by wiping with 70% ethanol between samples. Only symptomless plants were sampled: if a selected plant had symptoms, a neighbouring plant was sampled. After collection, plants were stored in a coldroom (ca. 4 °C) until processing.

Plant samples were put into conical flasks with sterile saline and 0.02% Tween and shaken for 30 min on a wrist-action shaker. The volume of saline was adjusted according to the size and number of plants in the sample (1 ml $plant^{-1}$ at the first sampling, 4 ml $plant^{-1}$ at the second sampling). After shaking, the extracts were diluted (10^{-1} and 10^{-2}) and 100 µl of the undiluted extract and of each dilution was spread onto plates of FS agar with a bent glass rod. Plates were incubated at 30 °C for 3 days and the number of typical *Xcc* colonies counted. Identities of the colonies were confirmed by sub-culture to YDC for comparison with the inoculated isolate and by slide agglutination. A number of colonies were also inoculated into cabbage cv. Wirosa to confirm pathogenicity.

Statistical analysis

The proportion of seeds infected was estimated using the STpro program (Ridout & Roberts, HRI, Wellesbourne, UK) and the mean number of bacteria per seed was estimated as a weighted mean, using the number of seeds in the sample as the weighting factor. The effect of treatments on the proportion of plants with visible symptoms, on the proportion of symptomless contaminated plants (as estimated by the proportion of positive samples) and on the mean numbers of bacteria per plant was studied using the generalised linear modelling facilities of Genstat V (Payne et al., 1993). Control (uninoculated) treatments were omitted from the analyses. The analysis was done with treatments specified as qualitative factors, to obtain an analysis of deviance equivalent to an analysis of

variance, and as quantitative variables, to obtain predictive equations.

The proportions of symptomless contaminated plants were estimated from the coefficients of the fully parameterised factor model fitted to the proportion of positive samples from the leaf washings. In some cases, all samples were positive, making the estimation of standard errors problematical (Ridout, 1994); 95% likelihood-based confidence intervals were therefore obtained using the STpro program.

Results

The amount of water applied for the two overhead watering treatments and the average compost moisture until 95% emergence for each watering regime are shown in Table 1. Compost was wetter in the capillary watering treatments than in the equivalent overhead watering treatment and plants grown on capillary matting were larger than those grown under overhead irrigation. Temperature in the glasshouse ranged from 11 to 30 °C with an overall mean of 16 °C for the 6 week duration of the experiment. There were no differences in germination between inoculation treatments or watering regimes.

Seed infection

The mean dose of bacteria received per seed and maximum-likelihood estimates of the proportion of seed infected/contaminated are shown in Table 2. The ratios of the estimated mean dose of bacteria received per seed approximately paralleled the ratios of the inoculum concentrations, and the estimated proportion of seeds infected/contaminated declined with inoculum dose.

Symptoms

Symptoms first became apparent on cotyledons 20 days after sowing, often as slight marginal necrosis with a chlorotic halo. As lesions progressed, veins became blackened and infected cotyledons wilted and eventually dropped off prematurely. *Xcc* was successfully re-isolated from all suspected lesions. The proportion of plants with symptoms in each treatment are shown in Table 3. Following the detailed assessment at 4 weeks after sowing, it became increasingly difficult to determine visually whether plants were infected at the later stages of the experiment due to the abscission of symptomatic cotyledons. Thus, when a limited final assessment of symptoms was done on completion of the experiment, the proportion of infected plants had apparently declined (data not shown). A decision was therefore made not to carry out a full assessment.

Table 3. Percentage of plants with black rot symptoms for each treatment 3 and 4 weeks after sowing, followed by approximate standard errors (from generalised linear model)

Watering regime[1]	Inoculum (cfu seed^{-1})			
	3.6×10^3	1.5×10^2	1.4×10^0	0
3 weeks				
HS	19.0 ± 2.3	1.0 ± 0.6	1.0 ± 0.6	0.0 ± 0.0
LS	18.5 ± 2.3	2.0 ± 0.8	1.1 ± 0.6	0.0 ± 0.0
HC	10.6 ± 1.8	2.8 ± 1.0	1.4 ± 0.7	0.0 ± 0.0
LC	9.6 ± 1.7	2.4 ± 0.9	1.0 ± 0.6	0.0 ± 0.0
4 weeks				
HS	53.6 ± 1.7	5.1 ± 0.7	3.5 ± 0.6	0.0 ± 0.0
LS	30.6 ± 1.5	4.5 ± 0.7	2.9 ± 0.6	0.0 ± 0.0
HC	14.1 ± 1.2	3.9 ± 0.6	2.2 ± 0.5	0.0 ± 0.0
LC	16.5 ± 1.2	3.8 ± 0.6	1.6 ± 0.4	0.0 ± 0.0

[1]HS – high frequency overhead spray; LS – low frequency overhead spray; HC – high frequency capillary; LC – low frequency capillary.

Table 2. Seed inoculation treatments

Code	Inoculum concentration (cfu ml^{-1})	Mean dose received $\pm$ s.e.	Proportion[1] infected/ contaminated	Lower limit	Upper limit
A	2.0×10^7	3580 ± 1130	1	0.78	1
B	4.0×10^5	146 ± 39	0.3	0.14	0.55
C	8.0×10^3	1.42 ± 0.3	0.079	0.03	0.17
D	0	0	0	0	0.001

[1]Maximum-likelihood estimate of proportion of seeds infected followed by upper and lower 95% confidence limits, obtained from STpro program.

A series of complementary log–log models of the form

$$\ln[-\ln(1-p)] = a + b_1x_1 + b_2x_2 + \cdots + b_nx_n,$$

where p is the proportion of plants infected, a and $b_{1,\ldots,n}$ are constants and $x_{1,\ldots,n}$ are candidate explanatory variables, were fitted to the data. Control (uninoculated) treatments were omitted from the analyses.

A series of factor-only models were fitted to obtain an analysis of deviance equivalent to an analysis of variance (Table 4) (Payne et al., 1993); significance tests were done on the basis of χ^2 values . It was clear that inoculum dose had the most significant effect on the proportion of plants with symptoms at both three and four weeks after sowing. At 3 weeks, watering type had only a slight effect, with a marginally significant interaction between watering type and inoculum dose. At 4 weeks, the watering regime was much more significant with watering type having a major effect, and watering level and the interactions between the two were also significant. Thus, the proportion of plants with symptoms increased significantly with increasing inoculum dose, was significantly greater for plants which were subject to overhead watering, and, at 4 weeks, was significantly greater in the higher level of overhead watering than in the lower, but was not affected by watering level in the capillary watered treatments.

A number of explanatory variables were fitted to the data (log of the mean dose of bacteria, $\ln d$; mean soil moisture to 95% emergence, *phi*; number of overhead water applications, *noh*) together with appropriate factors (watering type, *wtype*; watering level, *wlev*). Some terms were mutually exclusive in the models because of their intercorrelations, e.g. the variable *noh* is highly correlated with *wtype*, and *phi* is highly correlated with *wlev*. A summary of the best models is given in Table 5. The most important explanatory variable at both assessments was $\ln d$. (model 1). At 3 weeks, inclusion of a second term was significant but much less so than $\ln d$, and a model (model 2) with separate coefficients for $\ln d$ for each watering type was marginally better than including separate intercepts for each watering type (model 3) or a term for the number of overhead waterings (model 4). At four weeks, inclusion of a second term was highly significant, with inclusion of a variable for the number of overhead waterings (model 2) marginally better than separate coefficients for $\ln d$ (model 3) or separate intercepts for each watering type (model 4).

Leaf washings

Proportion of plants. The estimated proportion of infected/contaminated symptomless plants in each treatment and their confidence limits are shown in

Table 4. Analyses of deviance for qualitative effects of treatments on *Xanthomonas campestris* pv. *campestris* in cauliflower transplants

Source of deviance	Proportion of plants with symptoms[1]				Proportion of contaminated but symptomless plants[1,2]				Number of *Xcc* per plant[3]	
	3 weeks		4 weeks		2 weeks		6 weeks		2 weeks	
	df	Mean deviance	df	Mean deviance	df	Mean deviance	df	Mean deviance	df	Mean deviance
Inoculum level	2	110.2	2	716.3	2	22.6	2	28.8	2	1757.9
Watering type	1	10.7	1	299.2	1	0.9	1	44.7	1	1098.8
Watering level	1	0.1	1	54.1	1	0.7	1	5.0	1	6.4
wtype inoc[4]	2	4.3	2	17.4	2	2.0	2	4.0	2	150.0
wlev inoc	2	0.1	2	3.1	2	2.1	2	0.7	2	377.5
wtype wlev	1	0.4	1	32.2	1	0.1	1	7.6	1	2.0
wtype wlev inoc	2	0.3	2	4.7	2	0.3	2	0.0	2	679.5
Residual	156	1.0	660	1.2	84	0.7	84	0.7	84	181.3
Total	167	2.4	671	4.0	95	1.2	95	2.0	95	167.3

[1]Complementary log–log model.
[2]As estimated by the proportion of positive samples in leaf washings.
[3]Negative binomial model, $k = 1.33$.
[4]*wtype* – watering type; *inoc* – inoculum level; *wlev* – watering level.

Table 5. Summary of best models for the relationship between the proportion of plants with black rot symptoms, p, and various explanatory variables

Model no.	Model: $\ln[-\ln(1-p)]$[1]	df	Deviance	%[2]
3 weeks				
1	$-5.47 + 0.43 \ln d$	1	203.3	84.3
2 (separate coefficients for *wtype*)	$-5.46 + 0.46 \ln d$ (*wtype* = S)	2	217.9	90.4
	$-5.46 + 0.39 \ln d$ (*wtype* = C)			
3 (separate intercepts for *wtype*)	$-5.27 + 0.43 \ln d$ (*wtype* = S)	2	213.9	88.7
	$-5.74 + 0.43 \ln d$ (*wtype* = C)			
4	$-5.62 + 0.43 \ln d + 0.006 noh$	2	209.1	86.7
	Total for treatment stratum	11	241.1	
4 weeks				
1	$-4.63 + 0.42 \ln d$	1	1305.5	69.9
2	$-5.19 + 0.44 \ln d + 0.014 noh$	2	1659.0	88.8
3 (separate coefficients for *wtype*)	$-4.62 + 0.48 \ln d$ (*wtype* = S)	2	1624.2	86.9
	$-4.62 + 0.34 \ln d$ (*wtype* = C)			
4 (separate intercepts for *wtype*)	$-4.29 + 0.43 \ln d$ (*wtype* = S)	2	1597.1	85.5
	$-5.28 + 0.43 \ln d$ (*wtype* = C)			
	Total for treatment stratum	11	1868.4	

[1]Key to variables: d, dose received; *wtype*, watering type (S = overhead spray; C = capillary); *noh*, number of overhead waterings.
[2]% of deviance accounted for compared to full treatment model.

Table 6. Maximum-likelihood estimates and 95% confidence limits of percentage of symptomless cauliflower transplants contaminated/infected with *Xanthomonas campestris* pv. *campestris* for each treatment 2 and 4 weeks after sowing

Watering regime[1]	Inoculum (cfu seed^{-1})							
	3.6×10^3		1.5×10^2		1.4×10^0		0	
	%	Limits	%	Limits	%	Limits	%	Limits
2 weeks								
HS	78.7	11.0–100.0	4.5	1.2–14.0	2.6	0.6–8.1	0.0	0.0–1.3
LS	21.0	6.3–50.0	8.8	2.8–21.0	4.0	1.1–12.0	0.0	0.0–1.3
HC	78.7	11.0–100.0	1.2	0.2–3.9	1.9	0.5–5.2	0.0	0.0–1.3
LC	78.7	11.0–100.0	5.9	1.8–16.0	2.0	0.5–5.5	0.0	0.0–1.3
6 weeks								
HS	100.0	61.0–100.0	82.1	11.0–100.0	82.1	11.0–100.0	2.7	1.0–5.9
LS	40.9	14.0–75.0	82.1	11.0–100.0	21.0	6.4–50.0	0.0	0.0–0.8
HC	24.7	6.7–53.0	21.0	6.4–50.0	0.0	0.0–1.5	0.0	0.0–0.8
LC	21.4	5.8–47.0	6.3	1.0–7.7	1.2	0.2–3.8	0.0	0.0–0.8

[1]HS – high frequency overhead spray; LS – low frequency overhead spray; HC – high frequency capillary; LC – low frequency capillary.

Table 6. At the final leaf washing (6 weeks) the presence of the pathogen was detected in the overhead-watered uninoculated control treatment on the bench with the high level watering regime, indicating that spread had occurred from infected plants on the same bench.

Analysis of the proportion of infected/contaminated was done by fitting the complementary log–log model

$$\ln\left[-\ln(1-p)\right] = \ln(N) + a + b_1x_1 + b_2x_2 + \cdots + b_nx_n,$$

where p is the proportion contaminated/infected, N is the number of plants in the sample (defined as an offset in Genstat), a and $b_{1,\ldots,n}$ are constants and $x_{1,\ldots,n}$ are explanatory variables. Control (uninoculated) treatments were omitted from the analyses.

As for the analysis of symptoms, a series of factor-only models were fitted to obtain an analysis of deviance similar to analysis of variance (Table 4) (Payne et al., 1993). At the first leaf washing (2 weeks), the only factor with a significant effect was inoculum

dose. At the second leaf washing (6 weeks), watering type also became highly significant and more so than inoculum dose. Watering level and the interactions between watering type and inoculum dose and level were also significant, but marginally so. Thus, the proportion of symptomless plants infected/contaminated increased with increasing dose. At 2 weeks after sowing, it was unaffected by either watering type or level, whereas, at 6 weeks after sowing, it was significantly greater in the overhead watering treatments than in the capillary treatments and also at the higher level in the overhead treatments.

As for the analysis of symptoms, a number of explanatory variables were fitted to the data and a summary of the best models is shown in Table 7. The most important explanatory variable at 2 weeks after sowing was $\ln d$, and inclusion of further terms in the model did not give significant improvements. At 6 weeks, the number of overhead waterings (model 1) and watering type (model 2) were more important than $\ln d$ (model 3). Inclusion of a second term for $\ln d$ gave a significant improvement to the model (model 4), but inclusion of additional terms gave no further improvement.

Numbers of bacteria. The mean number of *Xcc* per symptomless plant 2 and 6 weeks after sowing

Table 7. Summary of the best models for the relationship between the proportion of symptomless plants contaminated/infected with *Xanthomonas campestris* pv. *campestris*, *p*, and various explanatory variables, 2 weeks and 6 weeks after sowing

Model no.	Model: $\ln[-\ln(1-p)]$[1]	df	Deviance	%[2]
2 weeks				
1	$-4.23 + 0.34 \ln d$	1	33.0	59.6
	Total for treatment stratum	11	55.4	
6 weeks				
1	$-3.29 + 0.063noh$	1	82.7	66.5
2 (separate intercepts for *wtype*)	-0.34 ($wtype = \mathrm{S}$)	1	71.4	57.4
	-3.29 ($wtype = \mathrm{C}$)			
3	$-3.48 + 0.35 \ln d$	1	57.3	46.1
4	$-4.25 + 0.045noh + 0.32 \ln d$	2	108.8	87.5
	Total for treatment stratum	11	124.3	

[1]Key to variables: d, dose received; *wtype*, watering type (S = overhead spray; C = capillary); *noh*, number fo overhead waterings.
[2]% of deviance accounted for compared to full treatment model.

Table 8. Weighted[1] mean and standard error of number of *Xanthomonas campestris* pv. *campestris* per symptomless cauliflower transplant, for each treatment, 2 and 6 weeks after sowing

Watering regime[2]	Inoculum (cfu seed^{-1})			
	3.6×10^3	1.5×10^2	1.4×10^0	0
2 weeks				
HS	$7.1 \pm 0.1 \times 10^5$	$1.3 \pm 0.1 \times 10^5$	$7.5 \pm 0.5 \times 10^4$	0.0×10^0
LS	$3.4 \pm 0.1 \times 10^5$	$1.1 \pm 0.1 \times 10^5$	$7.9 \pm 0.8 \times 10^4$	0.0×10^0
HC	$3.6 \pm 0.1 \times 10^5$	$4.7 \pm 0.4 \times 10^3$	$2.5 \pm 0.2 \times 10^4$	0.0×10^0
LC	$2.7 \pm 0.1 \times 10^5$	$6.9 \pm 0.7 \times 10^4$	$1.0 \pm 0.1 \times 10^3$	0.0×10^0
6 weeks				
HS	$3.4 \pm 0.3 \times 10^6$	$3.2 \pm 0.1 \times 10^6$	$2.1 \pm 0.1 \times 10^6$	$8.1 \pm 1.0 \times 10^4$
LS	$1.2 \pm 0.5 \times 10^6$	$6.3 \pm 0.6 \times 10^5$	$1.6 \pm 0.2 \times 10^6$	0.0×10^0
HC	$6.3 \pm 2.6 \times 10^1$	$1.8 \pm 0.1 \times 10^6$	0.0×10^0	0.0×10^0
LC	$2.5 \pm 2.5 \times 10^3$	$1.5 \pm 0.1 \times 10^6$	$9.0 \pm 1.1 \times 10^5$	0.0×10^0

[1]Values weighted by the number of plants in each sample.
[2]HS – high frequency overhead spray; LS – low frequency overhead spray; HC – high frequency capillary; LC – low frequency capillary.

are shown in Table 8. Inspection of the data suggested that, at 2 weeks, the numbers increased with inoculum dose, with little difference between watering regimes and at 6 weeks, the numbers were much greater in the overhead watering regimes with no consistent differences between inoculum doses. However, analysis of the mean numbers per plant was problematical. Attempts to fit Poisson models resulted in convergence failures or divergence during the iterative procedures. An examination of the relationship between log(variance) and log(mean) indicated that the data could follow a negative binomial distribution. The aggregation parameters, k, for the negative binomial distribution, where $v = m + m^2/k$ and v and m are the variance and mean, respectively, were therefore estimated by fitting the linear equation

$$\ln\left(\frac{v}{m} - 1\right) = \ln(k) + \ln(m)$$

to be 1.326 and 0.8796 for the first and second leaf washings, respectively.

These parameters were then used to fit generalised linear models with negative binomial distribution and logarithmic link functions of the form

$$\ln(m) = a + b_1x_1 + b_2x_2 + \cdots + b_nx_n,$$

where m is the number of bacteria per plant, a and $b_{1,\ldots,n}$ are constants and $x_{1,\ldots,n}$ are explanatory variables. Data were weighted by the number of plants in the sample and control (uninoculated) treatments were omitted from the analyses. It was now possible to fit models to the data for 2 weeks after sowing, but still not for 6 weeks and we presume that this is the result of a combination of extreme data and an underlying lack of any consistent pattern.

As previously, a series of factor-only models were fitted to the data for 2 weeks after sowing, in order to obtain an analysis of deviance (Table 4). Both inoculum dose and watering type had significant effects, so that the mean number of bacteria per plant increased with increasing dose and was greater for plants with overhead water than with capillary watering.

As before, a series of explanatory variables were fitted to the data for 2 weeks after sowing and a summary of the best models is shown in Table 9. The most important explanatory variable was $\ln d$ (model 1), and inclusion of a second term gave a significant improvement with separate intercepts for each watering type (model 2) giving a better fit than including a term for the number of overhead waterings (model 3) or separate coefficients for $\ln d$ for each watering type.

Discussion

It is clear from these results that the dose of bacteria per seed and the watering regime have an effect on both the proportion of plants with symptoms and the proportion of contaminated/infected but symptomless plants. Both symptoms and the proportion of contaminated/infected symptomless plants responded similarly to the treatments. Initially (2 weeks after sowing), the proportion of contaminated plants was influenced only by the dose of bacteria and there was little difference between the watering regimes, but by 3 weeks the proportion of plants with symptoms was greater for plants subjected to overhead watering. The effects of watering became more subtle with time so that, by 4 weeks after sowing, the proportion of plants with symptoms was even greater for plants with the most frequent overhead watering regime but with no differences between

Table 9. Summary of best models for the relationship between the mean number of *Xanthomonas campestris* pv. *campestris* per plant, m, and various explanatory variables, 2 weeks after sowing. Negative binomial variance, $k = 1.33$

Model no.	Model: $\ln(m)$[1]	df	Deviance	%[2]
1	$10.43 + 0.28 \ln d$	1	3054.0	43.4
2 (separate intercepts for *wtype*)	$10.67 + 0.32 \ln d(wtype = \mathrm{S})$	2	4100.2	58.3
	$9.54 + 0.32 \ln d(wtype = \mathrm{C})$			
3	$9.98 + 0.29 \ln d + 0.027noh$	2	3725.6	52.9
4 (separate coefficients for *wtype*)	$10.41 + 0.32 \ln d(wtype = \mathrm{S})$	2	3296.2	46.8
	$10.41 + 0.23 \ln d(wtype = \mathrm{C})$			
	Total for treatment stratum	11	7037.2	

[1] Key to variables: d, dose received; *wtype*, watering type (S = overhead spray. C = capillary); *noh*, number of overhead waterings.

[2] % of deviance accounted for compared to full treatment model.

levels in the two capillary watering regimes. Finally, at 6 weeks after sowing, the watering regime became more important than the dose of bacteria in determining the proportion of infected/contaminated but symptomless plants.

It should be noted that at the first leaf washing at 2 weeks, as symptoms were not yet apparent, the estimate of the proportion of infected/contaminated plants was a true total as it would have included those that subsequently developed symptoms. Whereas, at the final leaf washing at 6 weeks, the estimate of the proportion of contaminated/infected plants did not include plants with symptoms, however, it would have included some plants which showed symptoms at 4 weeks after sowing but had become apparently symptomless due to abscission of infected cotyledons/leaves. This may have been the reason why it became impossible to fit a satisfactory model to the data for the number of bacteria at 6 weeks.

The lack of differences in disease and contamination levels between the watering regimes at the early assessments and the major differences between the overhead and capillary watering regimes at the later assessments suggest that considerable spread of the pathogen and secondary infection was occurring in the overhead watering system with very little (if any) in the capillary system. It would seem reasonable to conclude that the differences in disease and contamination between the capillary watering regime and the overhead watering regimes was due to horizontal spread of the pathogen and new infections rather than a greater transmission rate from seed to seedling. Thus, transmission *per se* was estimated directly only in the capillary watering regimes or at the earliest assessment in the overhead watering regimes and transmission plus spread in the later overhead watering regimes.

The primary aim of this work was to examine the transmission of *Xcc* from cauliflower seeds to seedlings in a module plant raising system similar to that used commercially in the UK. Although, commercially, watering of module trays is invariably done using overhead watering systems, the capillary watering treatments were included to limit the confounding effect of pathogen spread and secondary infection. The results obtained justified this approach and it is apparent that considerable secondary spread of the pathogen can occur with an overhead watering system. Thus, for predictive purposes it would seem essential to include this secondary spread in any model, and we suggest that the most useful models are: Table 5, 4 weeks, model 2 for symptoms; Table 7, 6 weeks, model 4 for contamination.

The hypothesis of independent action is widely used for the interpretation of dose response data (Ercolani, 1984; Meynell and Stocker, 1957; Roberts, 1985; Roberts et al., 1996; Shortley and Wilkins, 1965), and suggests that each of the individual bacterial cells in the dose received is capable of multiplying in the host and giving rise to disease. The (one-hit) probability, ω, of an individual being effective (i.e. causing disease or, in the case of contamination, successful colonisation of the leaf surface) may be quite small and depends on its reaching a suitable site and, having arrived, multiplying sufficiently to induce symptoms or becoming detectable. The probability, p, of a seedling being infected (i.e. having one or more lesions) is given by (Peto, 1953; Meynell and Stocker, 1957)

$$p = 1 - \mathrm{e}^{-\omega d},$$

implying

$$\ln[-\ln(1 - p)] = \ln(\omega) + \ln(d),$$

which is the form of the models here. Thus, the values of the constants in the equations fitted here represent $\ln(\omega)$ the log of the one-hit probability of transmission or infection for a single bacterial cell in the dose. The probability of transmission of the pathogen is ~ 0.014 ($= \mathrm{e}^{-4.25}$ from Table 7, 6 weeks, model 4) and the probability of infection (symptoms) is ~ 0.0056 ($= \mathrm{e}^{-5.19}$ from Table 5, 4 weeks, model 2).

A priori, it would be expected that the coefficient for $\ln(d)$ would be 1 (Roberts et al., 1996). The values obtained here were all significantly less than 1, suggesting heterogeneity in the value of ω. This is consistent with the results obtained during the studies on transmission of pea bacterial blight (Roberts et al., 1996), where possible reasons for this heterogeneity are discussed.

In contrast to the results obtained for pea blight (Roberts et al., 1996; Roberts, 1992), there appeared to be no effect of compost moisture on transmission rate. This may be a result of one or all of the following factors: the smaller seed size of brassica; more rapid emergence; shallower planting depth; type of germination, i.e. epigeal; an artefact of the range of soil moistures examined being too narrow.

These results support the view put forward previously (Roberts et al., 1996) that the predictive power of seed tests may be improved by taking into account pathogen numbers when reporting results or designing

assays. Most seed assays for bacterial pathogens, as well as having a detection threshold in terms of the proportion of infected seeds in a seedlot, also have a detection threshold in terms of the inoculum load per seed which can be detected, clearly both of these aspects should be considered when designing assays.

Detection of the pathogen in the control (uninoculated) treatment in the high frequency overhead watering regime at the final assessment indicates that a separation distance of one tray width (0.4 m) would be inadequate to prevent spread of *Xcc* from infected to healthy seed lots. In commercial plant raising nurseries, modules sown with large numbers of different seedlots may be grown in a single glasshouse with little or no separation between different lots; clearly, there would be considerable potential for cross-infection or contamination. It is also likely that transplants infected/contaminated in this way would be symptomless.

In practical terms, these results indicate that the amount of overhead watering applied will have a major impact on the dissemination of the pathogen during transplant production in module trays. The results are also consistent with the suggestion put forward in the introduction that the change to centralised production of module transplants could account for at least some of the increased frequency of disease outbreaks in the UK and that a lower tolerance standard for seed infection may be necessary under these circumstances.

Acknowledgements

The authors would like to thank the glasshouse staff at HRI, Wellesbourne for their assistance in setting up and maintaining this experiment and K. Phelps for advice on the analysis of results. This work was funded by the Ministry of Agriculture Fisheries and Food (England and Wales).

References

Cook AA, Larson RH and Walker JC (1952) Relation of the black rot pathogen to cabbage seed. Phytopathology 42: 316–320

Ercolani GL (1984) Infectivity titration with bacterial plant pathogens. Annual Review of Phytopathology 22: 35–52

Lyons NF and Taylor JD (1990) Serological detection of and identification of bacteria from plants using a *Staphylococcus aureus* slide agglutination test. Plant Pathol 39: 584–590

Meynell GG and Stocker BAD (1957) Some hypotheses on the aetiology of fatal infections in partially resistant hosts and their application to mice challenged with *Salmonella paratyphi-B* or *Salmonella typhimurium* by intraperitoneal injection. J Gen Microbiol 16: 38–58

Miles AA and Misra SS (1933) The estimation of the bactericidal power of the blood. Hygiene, Cambridge 38: 732–749

Payne RW, Lane PW, Ainsley AE, Bicknell KE, Digby PGN, Harding SA, Leech PK, Simpson HR, Todd AD, Verrier PJ and White RP (1993) Genstat 5 Release 3 Reference Manual. Clarendon Press, Oxford

Peto S (1953) A dose-response equation for the invasion of micro-organisms. Biometrics 9: 320–335

Ridout MS (1994) A comparison of confidence interval methods for dilution series experiments. Biometrics 50: 289–296

Ridout MS (1995) Three-stage designs for seed testing experiments. Appl Statis 44: 153–162

Roberts SJ (1985) A note on *Pseudomonas syringae* pv. *berberidis*: comparative infection studies in attached and detached leaves. J Appl Bact 59: 369–374

Roberts SJ (1992) Effect of soil moisture on the transmission of pea bacterial blight (*Pseudomonas syringae* pv. *pisi*) from seed to seedling. Plant Pathol 41: 136–140

Roberts SJ, Ridout MS, Peach L and Brough J (1996) Transmission of pea bacterial blight (*Pseudomonas syringae* pv. *pisi*) from seed to seedling: effects of inoculum dose, inoculation method, temperature and soil moisture. J Appl Bact 81: 65–72

Schaad NW (1989) Detection of *Xanthomonas campestris* pv. *campestris* in Crucifers. In: Saettler AW, Schaad NW and Roth DA (eds) Detection of Bacteria in Seeds and Other Planting Material. American Phytopathological Society, St. Paul, USA, pp 68–75

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

Shortley G and Wilkins J (1965) Independent-action and birth–death models in experimental microbiology. Bacteriol Rev 29: 102–141

Williams PH (1980) Black rot: a continuing threat to world crucifers. Plant Dis 64: 736–742