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Key staff:	Dr S J Roberts, Plant Health Solutions Ltd Dr Lauren Branfield, Stockbridge Technology Centre Mrs S. R. Payne, Plant Health Solutions Ltd
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Industry Representatives:	Rob Richardson, Johnson's of Whixley Richard Haacker, East of Scotland Growers
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Name] Dr S J Roberts

[Position] Plant Pathologist

[Organisation] Plant Health Solutions Ltd

Signature ..... Date .....

[Name] Dr Lauren Branfield

[Position] Project Manager

[Organisation] Stockbridge Technology Centre

Signature ..... Date .....

Report authorised by:

[Name] Dr S J Roberts

[Position] Director

[Organisation] Plant Health Solutions Ltd

Signature ..... Date .....

[Name] Mr. Michael Langdale

[Position] Operations Director

[Organisation] Stockbridge Technology Centre

Signature ..... Date .....

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GROWER SUMMARY

Headline

A range of studies over two years and across a range of eight different edible and ornamental plant species reinforced the critical importance of using healthy (pathogen free) planting material (seeds, cuttings, plug plants, liners) for the management of bacterial plant diseases.

Background

There are more than 100 known bacterial plant pathogens that affect, or could potentially affect, UK crops. Despite much previous research, diseases caused by bacterial pathogens continue to cause economic losses to growers, particularly in field vegetables, hardy nursery stock and protected ornamentals. The options for control with plant protection products have always been limited, and it is likely that this will continue. For the majority of bacterial plant diseases the primary source of infection is likely to be the seed or propagation material. The use of clean starting material provides the best prospects of long-term sustainable control of bacterial pathogens in horticultural crops; the exclusion of the pathogen through the use of clean starting material avoids the need for secondary interventions with e.g. Plant Protection Products etc. This collaborative project between Plant Health Solutions (PHS), Stockbridge Technology Centre (STC), Warwick Crop Centre (WCC) and growers and primarily focussed on developing best practice for the deployment of such a strategy. For a number of high priority model bacterial pathogens, the prevalence of the pathogen in starting material was determined, the benefits of clean starting material was demonstrated, and epidemiological data was obtained to set health standards for starting material. We also examine the feasibility of novel methods to produce high-health planting material as a second-line defence, and examined the potential for resistance deployment where this seemed feasible.

Summary

Brassicas and Black Rot

- Seed testing revealed several seed lots infested with *Xanthomonas campestris* pv. *campestris* (*Xcc*)
- *Xcc* was detected in several batches of transplants, where traced these were found to be derived from infested seed.

- More than 50 crops/locations have been walked/examined and levels of black rot assessed. High levels of disease in the field were associated with known infested seed lots or transplants.
- The use of high-health transplants resulted in reduced levels of black rot and increased yields, compared to conventionally produced transplants in the same field.
- Hot water seed treatment resulted in significant reductions in disease levels in the field compared to crops derived from untreated seed of the same seed lot.
- Whether or not the previous (brassica) crop was infected with black rot had little impact on disease levels compared to seed infestation.
- The risk of significant disease developing as a result of field carry-over between crops appears small compared to the risk from seed infestation.
- Significant field outbreaks of black rot are likely to be the result of failure of seed (suppliers) to meet the recommended health standard of <0.005%.

Broccoli spear rot

- Despite inoculation of transplants with pathogenic strains, no disease developed in two years of field trials to screen for the resistance.
- We were able to demonstrate both seed to seedling transmission and spread of the pathogen during plant-raising.
- The relative importance of seed infestation in driving epidemics remains to be clarified.

Coriander and parsley bacterial blight

- *Pseudomonas syringae* pv. *coriandricola* was detected in three out of four seed lots tested. The levels detected exceeded the recommended health standard.
- Significant field outbreaks of black rot are likely to be the result of failure of seed (suppliers) to meet the recommended health standard.
- We were unable to isolate any specific bacterial pathogen from parsley field samples.

Cherry laurel and bacterial shot-hole

- Forty-four sub-samples (8 batches) of mother-plants/liners were tested at potting. The pathogen, *Pseudomonas syringae* pv. *syringae* (*Pss)* was detected in four batches with levels ranging from 0.5 to 2.7%.

- Follow-up of resulting crops indicated higher levels of disease in batches in which *Pss* was detected.
- Micro-propagation of *Prunus lauroceraus* cv. Otto Luyken was successful. Plants were established in tissue-culture and a batch was transferred to a commercial tissue-culture company for further multiplication. These were successfully weaned and grown on. Initial testing of potted plants indicates that they are pathogen-free.

Hardy Geraniums and Xanthomonas leaf spot

- *Xanthomonas hortorum* pv. *pelargonii* (*Xhp*) was detected in several batches of plug plants upon delivery to a production nursery.
- Spiking sample extracts with known numbers of *Xhp*, suggests that detection failures may occur when background bacterial populations are relatively high and pathogen populations low.
- BioPCR testing of plug plants indicated that many batches of plug plants could be infested with low numbers of *Xhp*, that were not detected by conventional plating.
- Infected batches of plants that were overwintered had high levels of disease in the following spring. Such batches of plants likely provide an important additional inoculum source for current year's material.
- Disease incidence increased very rapidly on the production nursery in June 2020. The estimated r-values (apparent infection rate) were 0.12 to 0.22 per day.
- In an experiment to compare the effect of irrigation systems on the rate of disease spread from a single point source, no spread of disease or pathogen was detected in plants grown under protection with sub-irrigation. For plants grown outdoors with overhead irrigation, disease incidence increased from 0.8% to 21.4%, and the pathogen was detected on all symptomless plants sampled. The r-value (apparent infection rate) was 0.031 per day.
- Based on the r-value from the spread experiment a health standard of <0.032% would need to be achieved in plug plants to ensure disease incidence did not exceed 10% by the end of a 6-month growing season.

Hedera and Xanthomonas leaf spot

- *Xanthomonas hortorum* pv. *hederae* (*Xhh*) was detected in several batches of liners at the point of delivery to the nursery.

- In an experiment to compare the effect of irrigation systems on the rate of disease spread from a single point source, no spread of disease or pathogen was detected in plants grown under protection with sub-irrigation. For plants grown outdoors with overhead irrigation disease incidence increased from 0.8% to 21.4%, and the pathogen was detected on all symptomless plants sampled. The r-value (apparent infection rate) was estimated at 0.036
- Based on the r-value from the spread experiment a health standard of <0.013% would need to be achieved in plug plants to ensure disease incidence did not exceed 10% by the end of a 6-month growing season.

Delphiniums and bacterial blotch

- Forty-four sub-samples of plug plants representing 14 batches from a range of suppliers were tested for *Pseudomonas syringae* pv. *delphinii* in 2020. The pathogen was not detected in any batches.
- No disease was detected in follow up of batches in production.

Novel Production System

- A quasi ebb and flood system for producing module transplants or plug plants using only sub-irrigation was trialled at a commercial plant-raising nursery.
- Brassica transplants in 345 module trays were successfully produced using the system and both plant-raiser and end-user grower were happy with the quality of the plants.
- The system needed less watering and feeding than conventional production.
- The resulting plants out-yielded conventionally produced plants of the same variety planted in the same field.

Financial Benefits

Production of high-health kale transplants using a quasi ebb-and-flood system resulted in a significant reduction in disease and concomitant yield increase of 2 to 6 t/ha in Cavolo Nero kale. Based on Defra price for curly kale of £3.40 per kg, this is equivalent to a value of £6,800 to £20,400 per ha. There are no Defra statistics for UK kale production, but if we assumed this to be 1,000 ha, then the national value of the gain would be £6 to £20 million. Perhaps more importantly reduction in losses and crop write-offs in the UK would reduce the need for and cost of imports.

Action Points

- Growers should question suppliers of seed and young plants on the health standards that have been applied and request assurances that those standards have been achieved.
- It is essential to quarantine and check bought-in plant material carefully and if necessary, consider additional laboratory testing.
- Consider production of modules, plug-plants, liners with a sub-irrigation system.
- Avoid or minimise the use of overhead irrigation in production.

SCIENCE SECTION

Introduction

There are more than 100 known bacterial plant pathogens that affect, or could potentially affect, UK crops. Despite much previous research, diseases caused by bacterial pathogens continue to cause economic losses to growers, particularly in field vegetables, hardy nursery stock and protected ornamentals. The options for control with plant protection products have always been limited, and it is likely that this will continue. For the majority of bacterial plant diseases the primary source of infection is likely to be the seed or propagation material. The use of clean starting material provides the best prospects of long-term sustainable control of bacterial pathogens in horticultural crops; the exclusion of the pathogen through the use of clean starting material avoids the need for secondary interventions with e.g. Plant Protection Products etc. This collaborative project between Plant Health Solutions (PHS), Stockbridge Technology Centre (STC), Warwick Crop Centre (WCC) and growers, and primarily focussed on developing best practice for the deployment of such a strategy. For a number of high priority model bacterial pathogens the prevalence of the pathogen in starting material was determined, the benefits of clean starting material was demonstrated, and epidemiological data was obtained to set health standards for starting material. We also examined the feasibility of novel methods to produce high-health planting material as a second-line defence, and examined the potential for resistance deployment where this seemed feasible.

The primary aim of the project was to improve the management/control of high priority bacterial diseases of horticultural crops primarily through the use of starting material with appropriate health standards based on sound epidemiological data, and by best-practice recommendations to achieve those standards.

Brassicas and black rot

Black rot of brassicas is caused by the bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*). *Xcc* is well-established as a seed-borne pathogen. Despite published standards for seed health and control recommendations available to growers and plant raisers, some growers have seen a recurrence of black rot in recent years. Theoretically, if the recommended standards were being applied industry-wide we should see a continual decline in the occurrence. The aim of this work is to understand the reasons for recent apparent control failures and demonstrate the value of high health seed/transplants. Most of the work is focussed on a case-study on a particular farm that had previous had problems with black rot.

Materials and Methods

Site visits

Visits were made to the case study farm on a number of occasions during the late summer, autumn and winter in 2020-21 and 2021-22. Fields containing brassica crops of interest were walked and the levels of (suspected) black rot disease incidence assessed. Where suspected black rot was observed, leaf samples were collected and taken to the laboratory for isolations and confirmation. Visits were also made to two plant-raisers and samples of (mainly symptomless) transplants collected.

Isolations from symptomatic tissues

Pieces of tissue about 2-4 mm^2, usually from the leading/advancing edge of lesions, and including a vein, were aseptically excised from the leaves and comminuted in a drop of sterile saline on a sterile microscope slide and observed under a light microscope using dark field illumination. Loopfuls of the resulting suspensions were then streaked on plates of Yeast Dextrose Chalk agar medium (YDC) or plates of FS or mCS20ABN selective media (Roberts & Koenraadt, 2005) when available.

Resulting bacterial colonies with appearance typical of *Xcc* were then sub-cultured and their identity confirmed by PCR (using XC and ZUP primers) and/or pathogenicity tests.

Seed testing

Specific seed lots that had been used to produce the crops observed in the field were obtained from the grower or plant-raiser or requested from the seed companies and up to 75,000 seeds tested for each lot.

The method for seed testing was as described in a previous project, FV 335 (Roberts, 2009), and based on an International Seed Testing Association (ISTA) validated method (Roberts & Koenraadt, 2005) with the addition of a centrifugation step to improve the analytical sensitivity (Roberts *et al.*, 2004). Briefly, sub-samples of up to 10,000 seeds were shaken in saline plus 0.02% Tween 20 for 2.5 h then diluted and plated on FS and mCS20ABN selective media. Following incubation, suspect colonies of *Xcc* were then sub-cultured and their identity confirmed by PCR and/or pathogenicity tests.

Transplant testing

In 2020, transplants were collected by the grower on arrival at the farm. In collaboration with the grower, a sampling plan for transplants was devised, the aim being to obtain a representative sample of each batch of transplants of interest upon arrival at the farm and before planting. Essentially this meant defining the numbers of plants to sample from each accessible module tray in the stillages, based on the expected batch size. Generally from each batch, six sub-samples of 48 plants were collected by the grower and sent to the PHS laboratory for processing.

A different approach to sampling and testing was followed in 2021, as we had access to transplants at the plant-raising nurseries. Samples were collected from nurseries prior to despatch to the growers to obviate previous issues with perceived contamination of foliage whilst in stillages. Testing was aimed at examining more different batches/varieties of transplants, but fewer sub-samples from each batch. At one nursery supplying the case-study farm we targeted batches of transplants of varieties where there had either been previous issues or where we had treated seed. At the other nursery we targeted varieties where *Xcc* had been detected in the previous year.

The method for transplant testing was as described in a previous project, FV 335 (Roberts, 2009). Transplants were stomached in a minimal volume of saline plus 0.02% Tween 20 and the resultant extract diluted and plated on FS selective media. Following incubation, suspect colonies of *Xcc* were then sub-cultured and their identity confirmed by PCR and/or pathogenicity tests.

The proportion of infested seeds and transplants was estimated by maximum likelihood methods using a stand alone computer program STPro (Ridout & Roberts, 1995).

High-health Transplants

In each year, a batch of high-health transplants were produced from tested and hot-water treated seed using the sub-irrigation system being trialled by a plant raising nursery (see later section).

In 2020 a batch of approx 5,000 transplants (15 trays) was delivered to the grower and planted in a 'squarish' block in a field surrounded by a planting of the same variety raised in the standard way by the grower's usual plant-raiser.

In 2021 a larger batch of approx 13,000 transplants (40 trays) was produced and most were planted in a single block in a field with blocks of the same variety raised in the standard way by the grower's usual plant-raiser.

Disease levels were monitored at intervals over the winter along with the surrounding crops. Shortly before the grower began harvesting in the fields, yield was assessed in three 'plots' in the centre of the high-health block and in three equivalent parallel plots in the surrounding crop of the same variety. The plots were spaced 10 m apart. Each plot consisted of a block of 3 m x 3 rows (5.4 m^2) and was expected to contain 27 plants. Plants were harvested in the same way as the commercial crop, with the whole kale head cut through the stem below the base of petioles of marketable blemish free leaves. If the initial cut was too low, i.e. unmarketable leaves were included (usually a result of *Xcc* infection), these were removed and the stem re-trimmed. Heads were shaken to remove excess water and then placed in a plastic crate. The crate plus heads were then weighed *in situ* in the field with a digital hand-held 15 kg balance (Kern, Switzerland) with a 20 g resolution.

The risk of in-field carry-over

To quantify the risk of in-field carry-over, a single block of 1,000 high-health kale transplants was planted (in August 2021) in an area where the previous crop had been heavily infected. This previous crop had a disease incidence of 100% and had been abandoned as not worth harvesting and ploughed-in in spring 2021. The block of kale transplants was surrounded by a Chard crop.

Results

Seed testing

Some 25 brassica seed lots (almost 100 sub-samples) were tested for *Xcc* (Table 1).

Xcc was detected in seven of these seed lots; estimated infestation levels in these positive lots ranged from 0.004% (1 in 25,000) to 0.2% (1 in 500). Unfortunately we were unable to obtain any seed of some lots of interest, and only limited quantities of others thereby

increasing the upper confidence limit of a negative test result (meaning that we can be less certain that the lot meets effective health standards). It should be noted that in seed lots where the pathogen was not detected, the upper 95% confidence limit is provided in the table.

Table 1. Summary of tests on brassica seed for *Xanthomonas campestris* pv. *campestris* (*Xcc*). Where all sub-samples were negative, the percentage infection (% inf) is the upper 95% confidence limit of a negative test.

Sample (Lot)	Type[a]	Cv	Source	N tested	N sub-samples	N positive	% inf
S2324	Kale CN	A	1	30,000	3	1	0.004
S2325	Kale CN	A	1	12,000	3	1	0.018
S2326+	Kale CN	A	1	75,000	9	0	<0.004
S2327	Kale CN	A	1	70,000	8	0	<0.004
S2328+	Kale CN	A	1	66,500	10	6	0.012
S2329	Kale	B	1	50,000	5	0	<0.006
S2330+	Kale	B	1	56,000	7	5	0.015
S2375	Kale	C	2	30,000	3	0	<0.01
S2589+	Broccoletto	G	5	20,000	4	2	0.014
S2590	Broccoli	H	6	10,000	2	0	<0.03
S2591	Cauliflower	J	2	2,000	2	0	<0.15
S2592	Cauliflower	K	7	4,000	2	0	<0.08
S2593	Cauliflower	L	4	5,800	2	0	<0.05
S2594	Cauliflower	M	4	4,500	2	0	<0.07
S2607	Kale CN	A	1	5,000	1	0	<0.06
S2608+	Kale CN	A	1	20,000	7	2	0.015
S2611	Russian Kale	R	9	12,000	3	0	<0.023
S3007	Savoy	S	1	9,500	6	5	0.200
S3008	Russian Kale	R	9	8,500	4	0	<0.035
S3009	Broccoli	H	6	5,000	1	0	<0.06
S3010	Kale	E	4	10,000	2	0	<0.03
S3011	Cauliflower	P	10	2,500	1	0	<0.12
S3012	Cauliflower	Q	6	1,500	1	0	<0.20
S3060	Kale CN	A	1	10,000	4	0	<0.03
S3062	Kale CN	A	1	5,000	2	0	<0.06

Notes:
[a] CN = Cavolo Nero

Transplant testing

In 2020, 35 sub-samples of transplants were tested for the presence of *Xcc*, representing six batches. Plants were sampled on delivery to the grower. *Xcc* was detected in two sub-samples from one batch, S2435 (Table 2), with an estimated infestation level of 0.7% and a mean no. of *Xcc* of up to 10^5 CFU per plant. A second sowing from the same batch of seed

was negative. Where all sub-samples were negative, the upper 95% confidence limit is shown in the table. In a number of sub-samples the background numbers of bacteria were very high (despite planting on selective media), making it very difficult to identify suspect *Xcc* colonies on the plates, and effectively reducing the analytical sensitivity of the test.

In 2021, the sampling/testing strategy was amended and we tested more batches of transplants but fewer sub-samples from each batch. Plants were also sampled at the nursery prior to delivery. Almost fifty batches of transplants from two nurseries were sampled and tested for the presence of *Xcc* (Tables 2 and 3). *Xcc* was detected in five batches. Three of these batches were different sowings from the same batch of seed. The estimated infestation levels in the positive batches was around 1 to 2%, with numbers of *Xcc* detected ranging from 5×10^2 to 3×10^5 CFU/plant. For the vast majority, *Xcc* was not detected. This does not mean that those other batches are necessarily free from *Xcc* as the number of plants sampled from each batch was relatively low, hence the level of infestation in the positive lots was in most cases less than the reliable detection limit (95% upper confidence limit) for the negative samples.

The theoretical analytical sensitivity of most tests was about 30 to 90 CFU/plant (the sensitivity varies according to volume of extraction buffer, which in turn depends on the size of the plants). Sampling of plants at the nursery rather than at delivery to the grower was generally successful in reducing the background numbers compared to 2020.

Table 2. Summary of tests on brassica transplants for *Xanthomonas campestris pv. campestris (Xcc*) at nursery 02. Where all sub-samples were negative, the percentage infection (% inf) is the upper 95% confidence limit of a negative test. The colony forming units per plant (CFU/plant) is the maximum value across all sub-samples. Seed lots which tested positive for *Xcc* are indicated with an asterisk (*). Hot-water treated seed lots are indicated by 'HW'.

Sample (Batch)	Type[a]	Cv	Seed lot	Source	N tested	N sub-samples	% inf	CFU/plant
Planted 2020								
S2402	Kale	D		3	280	6	<1	
S2404	Kale	E		4	192	4	<1.5	
S2405	Kale	F		3	280	6	<1	
S2415	Kale	C		2	280	6	<1	
S2435	Kale CN	A	S2328*	1	328	7	0.7	1.0E+05
S2442	Kale CN	A	S2328*	1	144	3	<2.1	
Planted 2021								
S2692	Kale	C		2	60	2	<5	
S2693	Kale	U		?	20	1	<15	
S2694	Kale	T		4	30	1	<8	
S2695	Kale	P		8	20	1	<15	
S2696	Kale	W		8	20	1	<15	
S2698	Kale CN	D		3	60	2	<5	
S2699	Kale CN	F		3	40	2	<8	
S2700	Broccoletto	G	S2589*	5	20	1	<15	
S2701	Kale	Q		?	60	2	<5	
S2702	Kale	Y		9	20	1	<15	
S2703	Kale	N		4	30	1	<10	
S2704	Kale	V		?	60	2	<5	
S2705	Russian	R		10	24	2	<13	
S2706	Broccoletto	G	S2589*	5	15	1	<20	
S2707	Savoy	S		1	20	2	<15	
S2759	Kale CN	A	S2328*	1	56	2	2.4	3.2E+5
S2760	Kale CN	A	S2608-HW	1	88	3	<3	
S2761	Kale CN	A	S2328-HW	1	116	4	<2.6	
S2762	Kale CN	A	S2328*	1	116	4	1	4.5E+4
S2763	Kale	B	S2330*	1	120	4	1	5.7E+2
S2764	Kale CN	X		3	29	2	<10	

Notes:
[a] CN = Cavolo Nero

Table 3. Summary of tests on brassica transplants for *Xanthomonas campestris* pv. *campestris* (*Xcc*) at nursery 01 in 2021. Where all sub-samples were negative, the percentage infection (% inf) is the upper 95% confidence limit of a negative test. Bacterial numbers as colony forming units per plant (CFU/plant) is the maximum value across all sub-samples.

Sample (Batch)	Type	Cv	Seed lot	Source	N tested	N sub-samples	% inf	CFU/plant
S2664	Savoy	23		13	30	1	<10	
S2665	Cauliflower	20		4	25	1	<12	
S2666	Cauliflower	10		7	30	1	<10	
S2667	Cauliflower	12		12	40	1	<8	
S2668	Cauliflower	6		13	25	1	<12	
S2669	Cauliflower	11		11	25	1	<12	
S2670	?	16		?	25	1	<12	
S2671	Cauliflower	19		9	30	1	<10	
S2672	Kale	21		4	30	1	<10	
S2673	Kale	17	S3010	4	30	1	<10	
S2727	Broccoli	22	S3009	12	60	2	<5	
S2728	Cauliflower	3		12	30	1	<10	
S2729	Cauliflower	8		4	30	1	<10	
S2730	Cauliflower	2	S3011	13	40	2	<8	
S2731	Cauliflower	13		13	20	1	<16	
S2732	Cauliflower	5		7	30	1	<10	
S2733	Cauliflower	15		13	30	1	<10	
S2734	Cauliflower	18	S3012	13	30	1	<10	
S2735	Cauliflower	7		12	30	1	<10	
S2736	Cauliflower	4		13	30	1	<10	
S2737	Savoy	14		2	55	4	1.9	2.8E+05
S2738	Cauliflower	1		11	15	1	<20	
S2739	Broccoli	22	S3009	12	15	1	<20	
S2740	Romanesco	9		13	20	1	<16	

Table 4. Summary of black rot disease levels (Max inf, the maximum incidence across all assessment dates)) and other details for some of the key crops followed Sept to Feb 2020-21. Hot water treated seed is indicated by 'HW' in the seed infestation column. Previous crops known to have been infected with *Xcc* are marked with an asterisk (*)

Field	Type[a]	Crop/Cv	Planted (wk)	Max inf (%)	Transplants Inf (%)	Seed inf (%)	Previous Crop
MT	Kale	W	32	0	nt	nt	Kale
MT	Russian	R	32	75	nt	nt	Kale
MT	Savoy	S	32	100	nt	0.20	Kale
MT	Brocoletto	G	32	75	nt	0.014	Kale
MT	Kale CN	A High Health	32	56	**	HW <0.004	Kale
MT	Kale CN	A 2019	33	90	<2.1	0.012	Kale
MT	Kale	B	33	0.1	nt	0.015	Kale
CL	Kale CN	D	28	0	<1	nt	Rye/Vetch
CL	Weed	Charlock-Far	na	100	nt	nt	Rye/Vetch
CL	Weed	Charlock-Near	na	0	nt	nt	Rye/Vetch
CL	Kale CN	F	29	70	<1	nt	Rye/Vetch
CL	Brocoletto	G	29	100	nt	0.014	Rye/Vetch
CL	Kohlrabi	KR Near G	29	100	nt	nt	Rye/Vetch
CL	Kohlrabi	KR Away G	29	0	nt	nt	Rye/Vetch
KD	Kale CN	A 2019	32	78	0.7	0.012	Kale*
FL	Kale CN	F	29	0.1	<1	nt	Cereal
FL	Kale	O	29	0	<1.5	nt	Cereal

Notes:
[a] CN = Cavolo Nero
nt = not tested
** We found a single bacterial colony on one plate, later identified as race 5, but race 5 was not detected in the crop.

Fields assessments 2020-21

More than 30 crops/locations were walked/examined and levels of black rot assessed on up to five occasions, over the period from September 2020 to March 2021. Varying levels of disease were observed in the fields from zero to effectively 100% incidence. Around sixty samples of symptomatic leaves were collected for isolation and confirmation. In most cases, symptoms suspected of being caused by *Xcc* in the field were confirmed by isolation in the laboratory. However, it was not always easy to distinguish between lesions associated with *Alternaria* spp. and those caused by *Xcc*.

In addition to the classic V-shaped lesions that develop from the edges of leaves, other less typical, less easily recognised symptoms were also observed:

- "classic" V-shaped yellow/pale brown lesions with black veins developing from the edges of leaves
- yellow/pale brown necrotic lesions in the middle of leaves (these can be confused with down mildew)

- yellow/pale brown necrotic leaf margins, particularly in curly kale (borecole) types
- water-soaked or pale papery lesions de-limited by veins, particularly soon after planting

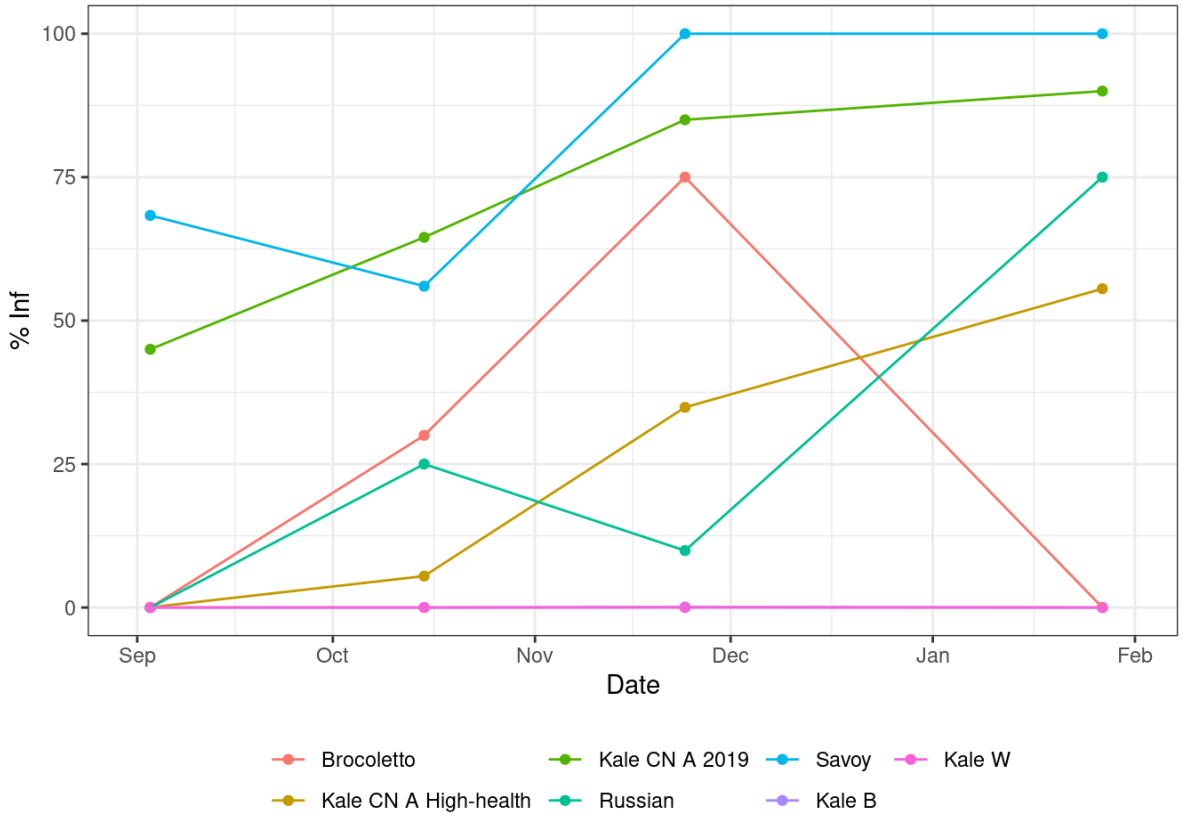


Figure 1. Development of black rot (disease incidence, % Inf) over time for several different brassica crops/varieties grown in the same field (MT) in 2020-21. For more details of each crop see Table 4.

A summary of some of the key crops followed in most detail is shown in Table 4. A graph showing the pattern of disease development in one field with multiple crops (MT) is shown in Fig. 1. Generally, crops grown from seed which was known to be infested had the highest levels of *Xcc* in the field regardless of whether or not the pathogen had been detected in the transplants. A Savoy cabbage (crop S) had the earliest and highest levels of infection in the field, the disease also appeared to be uniformly distributed. The seed lot had the highest levels of infestation detected in the study.

The cruciferous weeds charlock and shepherd's purse were also present in in some fields. typical *Xanthomonas* symptoms were seen on both and *Xcc* isolated. PCR-profiling and race-typing of these isolates suggests that they are different strains from those found in the surrounding/nearby crops.

Fields assessments (2021-22)

More than 28 crops/locations were walked/examined and levels of black rot assessed on up to five occasions, over the period from September 2021 to February 2022. Varying levels of disease were observed in the fields from zero to 77% incidence. More than 100 samples of symptomatic leaves were collected for isolation and confirmation. As in 2020-21, in most cases, symptoms suspected of being caused by *Xcc* in the field were confirmed by isolation in the laboratory. Again, it was not always easy to distinguish between lesions associated with *Alternaria* spp. and those caused by *Xcc*, and in some cases both pathogens were present in/on the same lesions. As in 2022-21 atypical water-soaked or pale papery lesions de-limited by veins, were seen early in the season, soon after planting.

A summary of some of the key crops followed in most detail is shown in Table 5. A graph showing the pattern of disease development in one field with multiple crops (GL) is shown in Fig. 2. As in 2020-21, crops grown from seed which was known to be infested had the highest levels of *Xcc* in the field regardless of whether or not the pathogen had been detected in the transplants. Thus Crop A 2019 U had the earliest and highest levels of infection in the field; the pathogen had been detected in the seed (0.012 %), and in the transplants (2.4 %).

As in 2020-21, typical *Xanthomonas* lesions were seen on the weed Charlock in some fields and *Xcc* isolated, again race-typing and PCR-profiling showed that the strains did not match the strains found in the surrounding/nearby crops.

Table 5. Summary of black rot disease levels (Max inf, the maximum incidence across all assessment dates) and other details for some of the key crops monitored for black rot caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*) during 2021-22. Hot water treated seed is indicated by 'HW' in the seed infestation column. Previous crops known to have been infected with *Xcc* are marked with an asterisk (*).

Field	Type[a]	Crop code	Planted (wk)	Max inf (%)	Transplants Inf (%)	Seed inf (%)	Previous Crop
MT	Kale CN	A (High Health)	32	0.80	<2.5	HW <0.004	Savoy*
MT	Russian Kale	R	30	23.5	<13	<0.023	Kale CN*
MT	Kale	Z2	30	0.04	nt	nt	Russian*
MT	Kale	W	30	0.94	<15	nt	Kale CN*
AHT	Kale CN	A (2019 U)	32	62.5	1	0.01	Grass
AHT	Kale	B	32	1.23	1	0.02	Grass
CL	Kale CN	F	30	0.00	<8	nt	Kale*
CL	Broccoletto	G	30	1.67	<15	0.01	Kale*
CL	Kohlrabi	KR	29	13.8	nt	nt	Kale*
CL	Kale	U	28	0.33	<15	nt	Kale*
CL	Kale CN	D	30	0.33	<5	nt	Kale*
CL	Savoy	S	30	24.1	<15	nt	Kale*
CL	Kale	W	28	0.00	<15	nt	Kale*
CL	Kale	RP	28	0.00	nt	nt	Kale*
FL2	Kale CN	A (2019 HW)	31	1.34	<2.6	HW <0.02	Kale
FL2	Kale	Var2	30	9.79	nt	nt	Kale
FL2	Kale	Var3	30	0.00	nt	nt	Kale
FL2	Kale	Var4	30	1.14	nt	nt	Kale
GL	Kale CN	A (2019 HW)	32	21.2	<2.6	HW <0.02	Kale
GL	Kale CN	A (2019 U)	32	76.9	2.4	0.01	Kale
GL	Kale CN	X	31	1.00	<10	nt	Kale
GL	Kale CN	A (2020 HW)	32	23.7	<3	HW <0.06	Kale
GL	Kale CN	A High Health	32	14.0	<2.5	HW <0.004	Kale
GL	Kale	O	32	0.00	nt	nt	Kale

GR	Kale CN	F	28	85.0	nt	nt	Leeks
GR	Kale CN	X	29	40.0	nt	nt	Leeks

Notes:
[a] CN = Cavalo Nero
nt = not tested

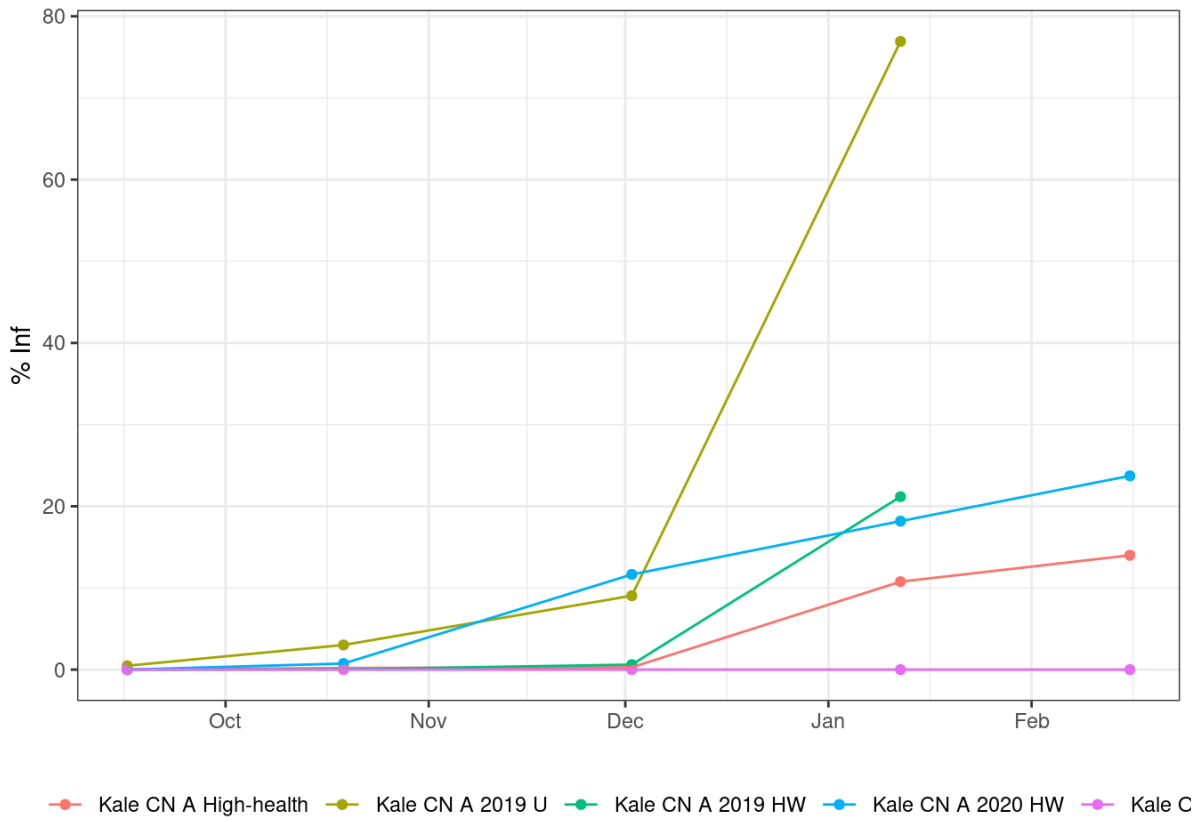


Figure 2. Development of black rot (disease incidence, % Inf) over time for several different brassica crops/varieties grown in the same field (GL) in 2021-22. For more details of each crop see Table 5.

High Health Transplants 2020-21

In the 2020 batch of 5,000 planted in August 2020, black rot did develop, apparently spreading in from the surrounding crop. However, disease developed later and levels were consistently lower than in the surrounding crop (see A High Health vs A 2019 in Fig 1). The first infection observed was a lesion developing from the edge of a leaf that had been subject to bird damage. Race-typing indicated that the pathogen race in the high-health block (race 4) was the same race as in the surrounding crop. Due to weather conditions, planting of the surrounding crop was delayed by a week compared to the high-health crop, weed control was less successful in the high-health crop than in the surrounding crop.

Yield assessments were done in late January and showed a significantly greater yield (by 34%) for the high-health compared to the surrounding conventional crop (Table 6).

Table 6. Summary of yield measurements for kale crops grown from conventional and high-health kale transplants, in 2020-21 and 2021-22 seasons.

Transplants	Yield (t/ha)	Max. disease incidence (%)
2020-21		
Conventional	5.5	90
High Health	7.4	56
s.e.	0.2	
2021-22		
Conventional	11.0	77
High Health	17.3	14
s.e.	0.6	

High Health Transplants 2021-22

In the 2021-22 season a larger (compared to 2020-21) block of ~12,000 transplants were successfully produced and planted in a single block (in field GL) together with blocks of conventionally produced transplants of the same variety. Disease did develop, but to much lower levels than in the conventionally produced crop of the same variety grown from untreated seed (see A High-Health vs. A 2019 U in Fig 2). All agronomic operations, and weed control efficacy in particular, were similar in all blocks across the field.

Yield assessments were again done mid Jan and showed a significantly greater (by 57%) yield for the high-health compared to the surrounding conventional crop (Table 6).

Risk of in-field carry-over

A maximum of eight infected plants was observed in the block of 1000 high-health transplants planted in the field where a previous infected savoy crop (Fig. 1) had been grown. The previous crop had 100% disease incidence and was abandoned as being not worth harvesting. Symptoms were first seen at the second assessment (in October 2021) and formed two discreet patches of five and three plants. In each case it appeared that there was a single primary infection that had spread to a few immediately neighbouring plants. There was no subsequent increase in disease incidence, and by February 2022 the symptomatic leaves had been lost and no disease was observed in the block.

Discussion

Seed tests have shown the presence of low levels of *Xcc* in several seed lots. Crops grown from these seed lots generally had high levels of disease in the field. However, there was one anomalous curly kale variety (B): although we did observe occasional symptoms in the field, they were difficult to find and remained low throughout the season in both years, but

the seed infestation level of 0.015% was comparable to levels in other varieties grown in the same fields and that resulted in field incidence approaching 100%. We also detected *Xcc* in the transplants in 2021. Preliminary inoculation tests suggest that the tissues are susceptible, but it would seem that this variety may have some level of field resistance.

Based on the high levels of disease observed in the field, we also strongly suspected that some of the seed lots that we were unable to test were also infested, e.g. cultivar F in field GR in 2021-22 with a previous crop of leeks. This crop had been abandoned as the high levels of black rot made harvesting uneconomic. Communication with a seed supplier regarding seed lots where we suspect that that they might be infested, but were unable to obtain further seed for testing, indicated that the seed had been tested for black rot. However, it appeared that the testing did not achieve the recommended standard (<0.005%) and the method used may not have achieved the required analytical sensitivity.

Tests on transplants identified the presence of *Xcc* in one batch in 2020 and four batches in 2021; four of these five batches were derived from seed lots which were known to be infested with *Xcc*, for the fifth we were unable to test the seed, but it seems very likely that it was infested. There were three batches (S2442, S2700 and S2706) derived from seed known to be infested that were negative, but given the numbers of plants tested and resulting detection limits, it is quite feasible that these batches were nevertheless infested at similar levels to those seen in the positive batches.

In 2020 the transplants were collected by the grower after delivery, in 2021 we collected transplant samples directly from the plant-raising nurseries. This generally resulted in lower background populations of bacteria giving greater confidence in the results.

Observations of disease progress in the field generally fell into four patterns of disease in different crops:

1. Initial and continuing low or negligible levels of black rot through the season.
2. Initial low levels which gradually increased through the season.
3. Initial low levels which rapidly increased early the season.
4. Relatively high initial levels which continued to increase through the season.

Pattern 1 was generally associated with crops grown in fields where all batches of transplants had tested negative, and we presume that the seed was also clean.

Patterns 3 and 4 were associated with seed lots known to be infested and/or detection in the transplants, or seed that we strongly suspect was infested (but we were unable to obtain seed to test).

Pattern 2 was associated with local spread in the field from neighbouring crops within the same field into crops with undetectable levels in the transplants or seed.

Race-typing of isolates from the field is giving further insights, but perhaps also creating a more confusing picture. In one case (Russian kale in Fig 1), the initial low level of disease was very patchy, and we presumed this was a result of local within-field spread from the neighbouring heavily infected Savoy cabbage crop. However, race-typing of isolates from the field indicated that the race in the Russian kale was race 5, whereas the race in the Savoy and all the other crops in the field was race 4, suggesting that infection may well have resulted from a low initial level of infection in the Russian kale transplants rather than spread in the field. However, more recent race typing of isolates from the Savoy seed lot has indicated that it contained multiple races, including race 5, even though only race 4 was detected in the Savoy crop.

In most cases, disease levels apparently declined at the later assessments in January and February. This was due to a combination of factors: few or no visible new infections developing during the colder months; secondary invasion of lesions/tissue by other bacteria, particularly after heavy frosts, making confirmation more difficult; senescence and loss of infected leaves.

In 2020, a batch of 5,000 high-health transplants was produced using sub-irrigation system The quality of the transplants was similar to conventionally produced plants. They were planted in August and followed over the winter until harvest. They produced an excellent crop with lower disease levels than the surrounding crop of the same variety derived from conventionally produced transplants. Although the high-health transplants did become diseased following apparent spread from the surrounding conventional crop, disease developed later and the maximum disease incidence was much lower (56% vs 90%). The high-health crop produced a much greater yield than the surrounding crop (7.4 vs 5.5 t/ha). It is possible that much of this yield increase may be due to the lower levels of black rot, but it may also be in part due to the delayed planting of the conventional crop. Conversely because of these different timings, the high-health crop had a significant weed issue due to a missed herbicide spray.

In 2021, a larger batch of high-health transplants was successfully produced using the sub-irrigation system. In order to scale-up we supported the polystyrene sheets on up-turned pots (rather than the pallets used in the first year). The quality of the transplants was similar to conventionally produced plants. These were planted in a single block in the same field as a crop of the same variety raised from conventionally produced transplants. Again black rot did develop, apparently spreading from the conventional crop in the same field, but much

later and to lower levels than the conventional crop (14% vs 77%). Again the high health crop produced a much greater yield than the conventional crop (17 vs 11 t/ha), confirming the results of the previous year.

A common concern with black rot in brassicas is the risk of in-field carry-over between crops. A number of the crops followed in this study were planted in fields or locations within fields where there had been a previous brassica crop that was known to be infected. Irrespective of the previous crop, the highest disease levels in the field were observed in crops derived from seed lots and/or transplants that were known to be infested or we suspect were infested but had been unable to test. To further examine the risk of carry-over we planted a block of 1000 high-health transplants in an area of one field where the previous crop had been written-off due to the high levels (100% incidence) of black rot. We observed just two primary infections in the block, which did not spread further than the immediately surrounding plants. If we assume that these primary infections resulted from in-field carry-over from the previous crop, it would seem that, whilst in-field carry-over is possible, the risk of significant disease development and crop losses is much lower than risk associated with seed infestation.

Conclusions

- High levels of black rot in the field were associated with seed infestation levels that exceed the recommended health standard of <0.005%.
- Although field carry-over between crops possible, the risk of significant disease development is much lower than for crops derived from infested seed.
- Cruciferous weeds are not a significant source of disease.
- It appears that some seed suppliers may not be conducting adequate testing. Growers should press seed suppliers for details of the seed health standard achieved and request that it meets the recommended standard.
- Hot water treatment of infested seed lots was effective in reducing disease levels in the field.

Broccoli spear rot

Broccoli spear rot or head rot is primarily caused by biosurfactant-producing pectinolytic strains of *Pseudomonas fluorescens* (spear rot bacterium, SRB). Previous work at Wellesbourne in the 1990s provided evidence of differences in resistance amongst broccoli varieties, but there is no information for current varieties. Previous work has also shown that the pathogen can be seed-borne, be transmitted from seed to seedling, and then survive on transplants/plants to crop maturity and cause disease. In order to set effective seed health standards, and understand the relative importance of seed vs. external sources, there is a need to understand the rate of spread of the pathogen during plant raising.

Materials and Methods

PAN medium

PAN selective medium was based on a previous (unpublished) medium, and consisted of *Pseudomonas* Agar F (PAF; Difco) containing amoxicillin (65 mg/L), clavulanic acid (15 mg/L) and natamycin (50 mg/L).

The growth and recovery of key pathogen strains was examined by plating dilution series on plates of PAN and non-selective PAF media.

Selection of varieties

Varieties were selected based on recommendations from the grower representative. Seed of each of the selected varieties was requested from the relevant seed companies.

Variety trial 2021

Seed of the selected ten varieties was sown in 345 module trays (two trays per cultivar) and transplants raised according to normal commercial practice by Specialist Propagation Services (Kirton, Lincs). One week before despatch to the grower, all transplants were inoculated by spraying with a suspension of a single known pathogenic strain of the spear rot pathogen (2949B-2). Growth from a plate of PAF medium was suspended in 10 mL of SDW (sterile de-ionised water) to give a turbid suspension. This initial suspension was then further diluted (3 mL added to 500 mL) and applied with a Matabi 5 L sprayer using an

Orange Evenspray nozzle at the lowest pressure consistent with even application. One litre was sprayed over a block of 20 trays, i.e. ~50 mL per tray, whilst moving the sprayer in all directions (i.e. up/down, down/up, left/right, right/left) to ensure uniform coverage.

Just prior to despatch, two plants (one from each tray) of each variety were sampled and sent to PHS for testing.

Transplants were planted by the grower in two sites following a randomised complete block design, with two blocks at each site. Each plot consisted of one bed x 15 m, with ten plots per block.

Shortly before harvest (end of October) the trial sites were visited and the levels of spear rot assessed in each plot.

Variety trial 2022

Seed of the selected ten varieties was sown in 345 module trays (two trays per cultivar) and transplants raised according to normal commercial practice by Specialist Propagation Services (Kirton, Lincs). Two batches of plants were sown, one in week 20, and one in week 23. One week before despatch to the grower, transplants were inoculated by spraying with a suspension of the spear rot pathogen following the same method as in 2021. Whereas in 2021 only a single strain was used, in 2022 half the plants were sprayed with the same strain as last year (2949B-2), and half the plants were sprayed with a recent isolate obtained from a crop in Scotland in 2020 (9954).

Just prior to despatch, one plant of each variety inoculated with each isolate (two from each tray) were sampled and sent to PHS for testing.

The two batches of transplants were planted by the grower at two sites in randomised blocks as in 2021.

Shortly before harvest (early October) the trial sites were visited and the levels of spear rot assessed in each plot.

Laboratory resistance assessment

Florets (10-15 mm diameter) were aseptically excised from broccoli heads and placed in wells of a 5 x 5 multi-well plate containing moist vermiculite. The florets were then inoculated with 50 µl drops bacterial suspensions using a pipette. At least five florets of each cultivar were inoculated with each isolate. A 50 µl drop of Manoxol wetter was applied to approx half of the florets prior to inoculation with bacteria. Following inoculation the florets were incubated for 4 days at 25°C in the dark. The presence of rotting was recorded in each floret.

Spread on transplants 2020

This experiment was done at Stockbridge Technology Centre (STC).

Broccoli seed was hand sown in 345 module trays of Levington F2 growing medium and set out in blocks of 15 (5 x 3) on two separate glasshouse benches in the same glasshouse at STC. The benches were separated by one empty bench. Each bench had different irrigation systems. On one bench a moving gantry overhead irrigation system had been set up comprising an array of 80° flat fan nozzles. This was intended to mimic the typical watering system used by commercial brassica plant-raisers. On the other bench a sub-irrigation system (as used for the high-health transplant production) was set up, comprising a layer of polystyrene sheets to provide a flat surface, a layer of polythene with raised edges to retain water, a layer of capillary matting and a top layer of TexR fabric, with water supplied via trickle tape.

Seeds were inoculated by vacuum infiltration with isolate 2949B-2. Three inoculated seeds were sown in the centre of one cell in one tray on each bench to provide a single point source of primary infection for each treatment (bench). The seeds had been inoculated with a known pathogenic strain by vacuum infiltration. The success of the inoculation was checked by extracting, diluting and plating a sample of inoculated seed as above. Transmission from seed to seedling was checked by separately sowing a small number of seeds and extracting, diluting and plating the resulting seedlings one week after sowing.

Transmission and spread on transplants 2021

This experiment was done at Stockbridge Technology Centre (STC).

Broccoli seed was hand sown in 345 module trays of Levington F1S growing medium. Each tray was sown with a single variety; the central 7 x 7 block of cells was sown with inoculated seed and the remaining surrounding cells were sown with healthy seed.

The experimental design consisted of five different varieties inoculated with two different pathogen isolates (as used in the field trial) and grown on two separate glasshouse benches in the same glasshouse at STC. The benches were separated by one empty bench. Each bench had a different irrigation system. On one bench a moving gantry overhead irrigation system had been set up comprising an array of 80° flat fan nozzles. This was intended to mimic the typical watering system used by commercial brassica plant-raisers. On the other bench a sub-irrigation system (as used for the high-health transplant production) was set up, comprising a layer of polystyrene sheets to provide a flat surface, a layer of polythene with raised edges to retain water, a layer of capillary matting and a top layer of TexR fabric, with water supplied via trickle tape.

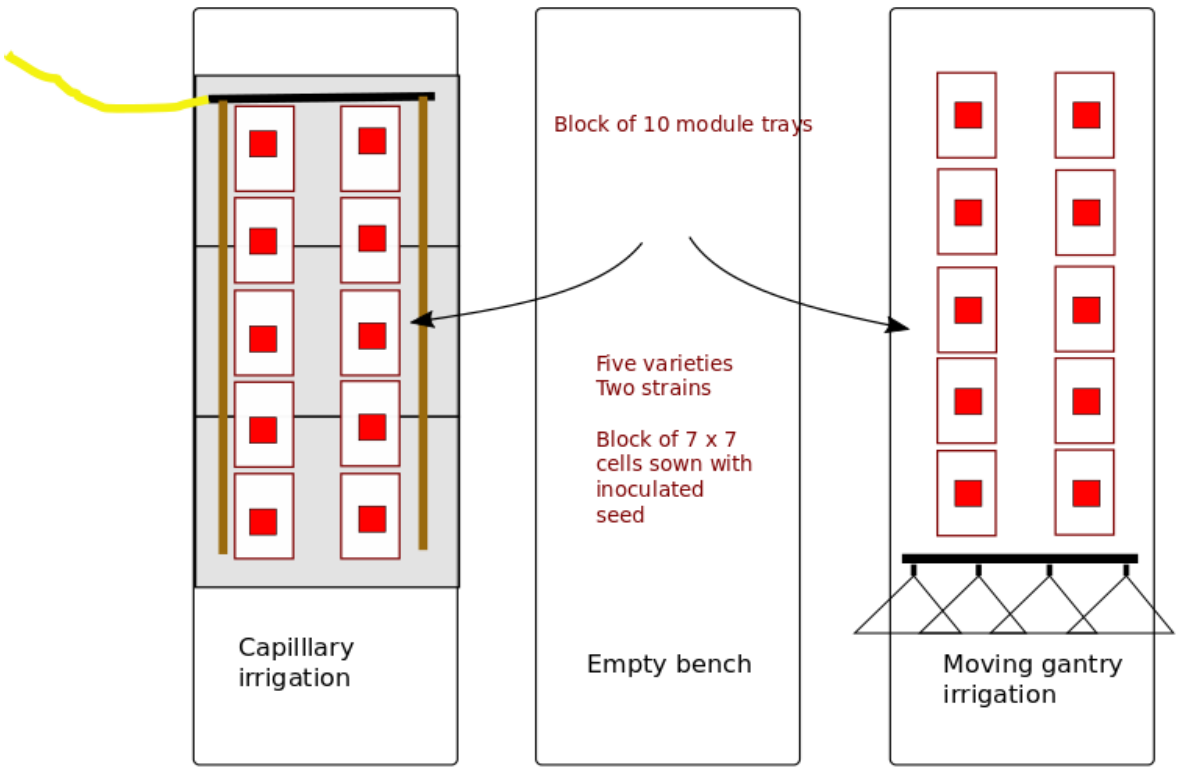
Sampling and detection on transplants

In 2020, samples were collected at two, four, and six weeks after sowing at approximately logarithmically increasing distances from the initial point source on each bench. Sampling distances and sizes were adjusted according to previous results. At both the first and last sample dates one of the seedlings at the point source was also sampled and tested.

In 2021, samples were collected at two, and five weeks after sowing and sent to PHS for testing. At two weeks, samples of five plants were collected from the central block (inoculated seeds) of each tray. At five weeks, three samples were collected from each tray: two from the outer cells (healthy seeds) at mean distances of approx 3 and 6.5 cells from the inoculated block and one from the central inoculated block.

Samples were sent to PHS for testing. Following receipt, samples were extracted in a minimal volume of sterile saline plus Tween by stomaching (or using a roller in the case of the smaller sample sizes). The extract was then serially diluted and plated as for seed testing. Suspect colonies were sub-cultured to sectored plates of PAF and compared to the inoculated strain. The identity of suspect isolates appearing similar to the inoculated strains was confirmed by PCR with specific primers.

Figure 3. Experimental layout for the broccoli spear rot transmission and spread experiment in 2021.



Results

PAN medium

The growth and recovery of two key strains of the target pathogen on the selective medium was confirmed.

Variety trial 2020

Testing of transplants prior to planting confirmed the presence of the inoculated strains with numbers in the range 10^2 to 10^4 CFU per plant.

Spear rot was observed in one plant at one of the two sites, out of a total of 5400 plants assessed. The strain isolated from the plant was not consistent with the inoculated strain.

Variety trial 2021

Testing of transplants prior to planting confirmed the presence of the inoculated strains with numbers in the range 10^3 to 10^5 CFU per plant.

No spear rot was observed in any of the plants at either of the two sites, out of a total of 5400 plants assessed.

Due to the absence of any symptoms on plants in the field, samples of heads were collected from each plot (3 per plot). One head was used for testing and inoculations and two heads were stored at room temperature to check for symptom development.

Rots developed in three varieties (A, D, H). In the case of rots developing on heads from plots inoculated with 2949B-2, attempts to re-isolate the original strain failed. In the case of the head from plot inoculated with 9954, we were able to re-isolate a strain with characteristics similar to the inoculated strain.

Attempts to detect the inoculated strains on symptomless harvested head failed in the case of plots inoculated with 2949B-2; in the case of plots inoculated with 9954 we were able to re-isolate strains with characteristics similar to the inoculated strain in two out of five cases.

In vitro laboratory assessment of resistance

In vitro assessment of tissues resistance did not give consistent results, with the relative ranking of varieties changing from experiment to experiment.

Spread in transplants 2020

The presence of the inoculated strain was confirmed on the seed, and transmission from seed to seedling was also confirmed on seedlings tested 7 d after sowing.

At the first sampling date (2 weeks after sowing), the inoculated strain was detected on seedlings from the primary infector cell on both the capillary and overhead benches, but in no other samples.

At the second sampling date (4 weeks after sowing), the inoculated strain was not detected in any samples (the infector cell was not sampled)

At the final sampling date (6 weeks after sowing) the inoculated strain was again not detected in any samples (the infector cells were sampled).

Transmission and spread on transplants 2021

The inoculated strains were detected in all of the plant samples collected two weeks after sowing from the central block sown with inoculated seed. Results for samples collected at five weeks after sowing are summarised in table 7. At five weeks the inoculated strains were detected in the majority of samples regardless of distance in almost all trays, and with little difference between the capillary irrigated and the overhead irrigated blocks of plants.

Table 7. Detection of spear rot pathogen strains in brassica transplants in module trays five weeks after sowing at different distances from cells sown with inoculated seed in 2021.

Watering system	Isolate	Distance (cells)	No plants per sample	No of samples	No positive
Sub	2949b-2	0	4	5	4
Sub	2949b-2	3	10	5	3
Sub	2949b-2	6.5	20	5	3
OH	2949b-2	0	4	5	5
OH	2949b-2	3	10	5	5
OH	2949b-2	6.5	20	5	4
Sub	9954	0	4	5	4
Sub	9954	3	10	5	4
Sub	9954	6.5	20	5	5
OH	9954	0	4	5	2
OH	9954	3	10	5	3
OH	9954	6.5	20	5	4

Discussion

The absence of any disease in two consecutive field trials was extremely disappointing. This was despite inoculation of the transplants, an approach which was successful in previous trials at Wellesbourne. In 2020 it was thought possible that the pathogen strain was the problem, therefore a second strain (obtained from an infected crop in Scotland in 2020) was used for the trial in 2021, when it was also split across two different planting dates to expose the crops to different weather conditions.

Given the lack of results from the field, we also attempted *in vitro* screening of resistance. The results obtained were inconsistent. This may have been due to the limited amount of material tested (in each experiment coming from a single head), and we suspect also due to variations in the relative 'maturity' of heads from experiment to experiment.

Our attempts to recover the strains inoculated onto the transplants from the heads met with very limited success. This is perhaps consistent with the lack of spear rot symptoms in the trials: if the pathogen had disappeared or populations have declined to low levels it is perhaps no surprise there is no disease. Thus our failure to observe disease in the trials could be due to a failure of the inoculated bacteria to survive or maintain high populations. This is in contrast to previous results at Wellesbourne, identifying the reason(s) for these inconsistencies could be of great value for the long-term management of this disease.

In the 2020 experiment at Stockbridge, we failed to detect any spread of the pathogen from the initial primary infection, although we did confirm that seed to seedling transmission was possible. The 2020 experiment was designed to examine spread over relatively longer distances (i.e. between multiple trays). In order to try and understand the reasons for this failure we conducted a second experiment in 2021 examining spread over shorter distances and with a higher initial level of inoculum. The 2021 experiment again clearly demonstrated that transmission of the pathogen from seed to seedling is possible. The experiment also demonstrated that spread of the pathogen can occur during plant raising, certainly within trays. This is in marked contrast to the results from 2020, where no significant secondary spread was detected. Again, identifying the reason(s) for these inconsistencies could be of great value for the long-term management of this disease.

Conclusions

- The spear rot pathogen can be transmitted from seed to seedling, and can spread during plant raising.

Coriander and parsley bacterial blight

Coriander bacterial blight is caused by *Pseudomonas syringae* pv. *coriandricola* (Psc). There have also been reports of a similar disease on parsley. It is seed-borne and seed testing methods and recommended seed health standards were devised by the author during an earlier HDC-funded project (FV 318) (Green & Roberts, 2010): <0.03% with an analytical sensitivity of 900 CFU. Commercial seed treatments are also available. If these standards were being applied throughout the industry it would be surprising to see any significant disease outbreaks (see https://planthealth.co.uk/articles/how-clean-is-your-coriander-seed/), nevertheless growers continue to report losses. It could be that either the standard is not being applied or it is inadequate, or if seed treatments are being used that these are not effective or are not being evaluated. A first step in understanding the current situation is to evaluate the levels (if any) in commercial seed stocks.

Material and methods

Samples of coriander seed were requested from growers via the Field Vegetables panel member for herbs.

Up to 9,000 seeds from each seed lot were tested for Psc as sub-samples of up to 3,000 seeds using the standard methods described in (Green & Roberts, 2010).

The proportion of infested seeds was estimated by maximum likelihood methods using a stand alone computer program STPro (Ridout & Roberts, 1995).

Observations and isolations were made from parsley leaf samples.

Results

Four seed lots were received and tested. Three of the four lots were positive for Psc with infestation levels greater than 0.015% and with mean pathogen numbers ranging from 2.4 to 3.7 CFU per seed for positive sub-samples (Table 4). Where all sub-samples were positive the lower 95% confidence limit is shown in the table, where all were negative, the upper 95% confidence limit is shown.

Disease symptoms were reported in the field for crops grown from the infested seed lots.

Table 8. Summary of tests on coriander seed for the presence of *Pseudomonas syringae* pv. *coriandricola (Psc)*.

Sample/lot	Grower	N tested	N sub-samples	% inf	CFU/seed	Crop symptoms
S2394	1	9,000	3	0.04	3.7E+02	Yes[1]
S2439	2	9,000	3	>0.02	2.4E+02	Yes[2]
S2440	2	9,000	3	<0.03		Uncertain[2]
S2441	2	9,000	3	>0.02	2.8E+02	Yes[2]

Notes:
[1] Confirmed by laboratory isolation.
[2] Grower observations.

Parsley leaf samples from three crops suspected as being bacterial were received. When observed under the microscope lesions from two of the three samples had typical Septoria pycnidia and conidia present. Septoria was not observed in lesions from the third sample and larger numbers of bacteria were present. Bacterial isolations were attempted onto both a non-selective medium and a medium selective for *Pseudomonas syringae*. Cultures were mixed, with no consistent type present, and no *Pseudomonas syringae* like strains present.

Discussion

Coriander bacterial blight is well-established as primarily a seed-borne disease, and a seed health standard was established in a previous HDC project (FV 318, Green & Roberts). The main question we sought to answer was whether this standard is adequate. Despite the limited number of seed lots tested, three out of four failed to achieve the recommended health standard, and significant disease was reported in the field crops grown from these lots.

The parsley samples (received as suspected bacterial disease) were predominantly affected by Septoria. In the one sample where large numbers of bacteria were present in lesions, nothing consistent was isolated and the mixed cultures were consistent with secondary invaders of already damaged tissues.

Conclusions

- Disease outbreaks in the field are associated with seed that does not meet recommended health standards.

Cherry laurel and bacterial shot-hole

Bacterial leaf spot and shot-hole of cherry laurel in the UK is caused by *Pseudomonas syringae* pv. *syringae* (*Pss*). As a vegetatively propagated crop, it is very likely that the primary source of the pathogen is the propagation material itself. The aim will be to conduct case studies to determine the prevalence of the pathogen on stock plants and bought-in plant material, and relate these to disease levels later in production, thereby providing an indication of the potential for disease control through the use of clean planting material.

In vitro micro-propagation has the potential to provide young plant material that is pathogen-free. We have identified a commercial micro-propagation company that have indicated a willingness to take on and potentially maintain material. The economics of cherry laurel production means that we would not expect that *in vitro* produced plants would be used by growers directly for production. However, we see the value of *in vitro* produced plants as providing a nucleus of high-health mother plants, that would then be used for conventional propagation via cuttings. The main questions then become: can they be maintained pathogen-free and for how long?

Materials and methods

Nursery visits, sampling and testing

Initial visits were made to two production nurseries for planning purposes and in one case to collect initial samples. At one nursery, a sampling scheme was devised for collection of samples from liners at the time of delivery/potting into final containers, and based on the expected numbers of plants in each batch. Samples consisting of six sub-samples of 40 leaves were collected by the nursery from each batch and sent to PHS for testing.

On arrival at the laboratory, sub-samples were transferred to stomacher bags, a minimal volume of saline plus Tween added and the bag manipulated to ensure that all leaves were wetted. Samples were then allowed to stand at room temperature for up 30 min before stomaching. Extracts were then diluted serially and plated on mP3 and MS3 selective media (Roberts, 2013a). Suspect colonies were then sub-cultured and identity confirmed using the GATTa tests and/or pathogenicity on lilac (Roberts, 2013a).

A follow-up visits was made to one nursery to assess disease levels in material that had been tested at potting. Samples were collected and isolations attempted to confirm the cause of symptoms.

Micropropagation

Terminal shoots of current growth, consisting of three or four nodes, were cut from apparently healthy plants of cv. Otto Luyken growing in the author's garden. These plants had never shown any shot-hole symptoms. The shoots were washed in running tap water. Leaves were then removed and shoot tips about 3-4 cm long were disinfected first in 70% iso-propanol then 0.3% chlorine plus 0.02% Tween and rinsed in SDW. Buds were then aseptically dissected out and placed on the surface of M&S proliferation medium in universal bottles, and incubated at 18 to 25°C with 14 h day length.

M&S proliferation medium consisted of Murashige and Skoog basal medium with sucrose (30 g/L) and supplemented with the phloroglucinol (0.161 g/L), benzyl amino purine (BAP, 2 mg/L), and indole butyric acid (IBA, 0.1 mg/L) (Sulusoglu & Cavusoglu, 2013).

Established cultures were sub-cultured approximately every two to three months, by aseptically dividing into individual shoots and replacing on fresh culture medium.

Following five or six rounds of sub-culturing/multiplication, a proportion of the larger shoots were transferred to rooting medium when sub-cultured. Rooting medium consisted of the same base medium but with the omission of BAP and the concentration of IBA increased to 0.5 mg/L (Sulusoglu & Cavusoglu, 2013).

A proportion of the explants were also transferred to a commercial micro-propagation company (Gentech) for further multiplication and weaning. Plants were weaned in 104 Jiffy 7 trays and leaf samples tested for the presence of Pss. Following weaning they were transferred to a commercial nursery for growing on under protection and separate from any other Prunus.

Results

Sampling and testing

Pathogenic *Pss* was detected in stock plants and several batches of plants (liners) at the time of potting (see Table 9). Where the pathogen was not detected, the upper 95% confidence limit is shown in the table.

Visible disease symptoms were observed in several batches of plants derived from the tested liners (see Table 6). Symptoms were confirmed as being caused by *Pss* by isolation.

Table 9. Summary of tests on cherry laurel batches for the presence of *Pseudomonas syringae* pv. *syringae (Pss).*

Sample/batch	Date	Supplier	Cv	Stage	N sub-samples	Total	% Inf
S2344	12/02/20	1	Rotundifolia	Stock	6	180	<1.7
S2345	12/02/20	1	Novita	Stock	3	90	1.3
S2343	07/02/20	2	Rotundifolia	Liner	6	240	<1.3
S2346	03/03/20	3	Zabeliana	Liner	6	240	1.7
S2347	03/03/20	3	Otto Luyken	Liner	6	240	2.7
S2349	17/03/20	4	Lusitanica	Liner	6	240	<1.3
S2395	03/07/20	4	Rotundifolia	Liner	6	240	0.5
S2396	03/07/20	4	Rotundifolia	Liner	5	200	<1.5

Table 10. Summary of follow-up assessments of bacterial shot-hole disease incidence (% Inf) and isolations of the pathogen *Pseudomonas syringae* pv. *syringae* (*Pss*) in cherry laurel production derived from tested liners.

Sample (Batch)	Cv	Open/Protected	% Inf	*Pss* isolated	Derived from:	Original % inf
S2443	Lusitanica	Open	2.1	yes	S2349	<1.3
S2444	Zabeliana	Open	55.4	yes	S2346	1.7
S2445	Otto Luyken	Open	24.6	yes	S2347	2.7
	Rotundifolia	Open	<0.2	no	S2343	<1.3
S2446+7	Rotundifolia	Protected	<0.13	no	S2395	0.5
S2448+9	Rotundifolia	Protected	<0.2	no	S2396	<1.5

Micropropagation

Explants were successfully established in culture. Depending on size, explants generally produced 3 to 6 shoots at each sub-culture. When transferred to rooting medium the majority of explants produced roots.

Explants were successfully multiplied and weaned by the commercial tissue-culture company. *Pss* was not detected on leaf samples from the weaned plants. About 800 plants were transferred to a grower in mid April 2021. Plants were potted on into 9 cm round pots in late June 2021.

About six hundred of the plants were transferred to Wellesbourne in the trays and immediately potted on arrival for use in the disease spread experiment. The majority of the plants at Wellesbourne failed to establish after potting and gradually died. Most plants failed to grow any new roots into the growing medium and the leaves turned brown from the tips progressing towards the petiole and main stem, eventually the stem also turned brown. This meant we were unable to carry out the planned spread experiment.

On the other hand the 200 plants retained by the grower have established and continued to grow. Both sets of plants were potted into the same potting mix and pot size, the primary difference between the plants being the water used for irrigation.

Discussion

The results of tests on mother plants and liners indicated that parental material can be infested with *Pss*, but that not all material is infested (or at least at levels below the sensitivity/detection limit of the test). This gives some confidence that a clean start approach to control of bacterial shot-hole could be feasible.

The follow-up observations of production material and the disease levels observed were entirely explainable based on the levels detected in the liners, their location, and production conditions. Thus the two batches with the highest levels detected in the liners, subsequently had the highest levels of disease symptoms later in the year when grown in the open. Batches with low or undetectable levels of *Pss* in liners had the lowest levels in production, and further modified by production conditions. Thus the low level in batch S2395 did not result in visible disease in subsequent production under protection.

We have successfully established cv. Otto Luyken in micropropagation, as a first step to production of high-health stock plants. These plants were then successfully weaned, potted on and remained pathogen-free. The explants are rather slow growing, and seem sensitive to the growing conditions and we suspect particularly to the water quality.

Hardy Geraniums and Xanthomonas leaf spot

Bacterial leaf spot of geraniums is caused by *Xanthomonas hortorum* pv. *pelargonii* (*Xhp*). There is very little prior information on the epidemiology of this disease.

Initial work was aimed at determining the prevalence of *Xhp* on plug plants and bought in plant material. Work in the second year continued work to identify sources of infection and understand the sensitivity of test methods. An experiment to examine the rate of disease spread from a single point source was undertaken as a first step to enable us to devise plant health standards.

Materials and methods

Preliminary experiments were done to confirm the growth and recovery of *Xhp* on potential selective media.

Detection in plug plants

Following an initial nursery visit, a sampling scheme was devised for collection of samples from plug plants based on the expected numbers of plants in each batch. Samples were collected from each batch at the point of delivery to the nursery, whilst still in Dutch trolleys.

In 2020, samples consisting of six sub-samples of 40 leaves were collected by the nursery from each batch and sent to PHS for testing. In 2021 samples consisting of one or more sub-samples of up to forty leaves were collected by PHS from each batch and transported to the PHS lab for testing.

Sub-samples were stomached in saline plus tween, diluted and plated on two selective media: BCBC (Holcroft & Roberts, 2002) and XanD (Lee *et al.*, 2009). Suspect colonies were sub-cultured to sectored plates of YDC, and their identity confirmed by PCR with specific primers or pathogenicity tests.

In 2021, in an attempt to study and improve test sensitivity we also prepared spiked samples, where we added a known number of *Xhp* to a sub-sample of some extracts and plated in the same way as samples. In addition for each sample we prepared an additional spread plate for the undiluted extracts and performed a plate-wash after 24-48 h incubation with 1 mL of saline. These plate wash extracts were then subject to PCR: bioPCR.

In a further attempt to understand infection sources, several batches of plugs were split immediately after delivery and sampling, with half going to a nursery site with no recent history of Geranium production and half remaining on the usual site.

Disease in production

Several visits were made to the nursery to follow-up on plants raised from the tested plug plants. At each visit the incidence of bacterial leaf spot was recorded in each batch and samples collected for isolation in the laboratory and confirmation that the symptoms were caused by *Xhp*.

Spread experiment

A batch of *Geranium himalayense* plants was propagated (by division) from parental plants with no previous history of disease. Leaf samples were also tested for the presence of *Xhp* (as for plug plants) prior to the start of the experiment. Plants were potted into 9 cm square pots of Levington CNS growing medium plus CRF and set out in two square blocks. One block (11 x 11 = 121 plants) was set out outdoors with an overhead irrigation system and the other block (8 x 8 = 64 plants) was set out in a polytunnel with a sub-irrigation system similar to that used for the high health brassica transplants. Polystyrene sheets were set out on top of pallets and a raised lip created around the edges with 2 cm wide strips of 7.5 mm foam insulation. The polystyrene sheets were then covered with a layer of polythene, followed by a layer of capillary matting and a top layer of Tex-R fabric. This fabric is coated with SpinOut®, a copper-based compound that inhibits rooting into the matting. The matting and fabric were allowed to overlap the edge at one end with sufficient excess to reach the floor. Trickle tape was then laid across the length of the area and connected to a header pipe. The header pipe was in turn connected to the irrigation system via a filter and pressure reducing valve. Watering was controlled by a battery-powered irrigation timer connected to solenoid valves.

A single plant inoculated with *Xhp* was placed in the centre of each block. The development of disease was then monitored at regular intervals (approx. weekly) and the location of diseased plants recorded on a map.

Data Analysis

The proportion of infested plug-plants was estimated by maximum likelihood methods using a stand alone computer program STPro (Ridout & Roberts, 1995).

Data from the spread experiment were analysed using R (R Core Team, 2021). The disease maps were used to generate Cartesian coordinates (x,y) for each plant and its health status at each assessment date. Models were then fitted to the data using

generalised linear modelling procedures (glm, binomial, logit link function) or non-linear modelling procedures (Levenberg-Marquardt type fitting algorithm).

Results

The growth and recovery of a recent isolate of *Xhp* was confirmed on the selective media. Recovery was also confirmed in a spiked sample when the first batch of plants were tested.

Detection in plug plants

In 2020, thirty-five sub-samples of plug plants were tested, representing seven batches and three suppliers, over the period April to July. *Xhp* was not detected in any of the batches (Table 11).

Table 11. Summary of tests on batches of Geranium plug plants for *Xanthomonas hortorum* pv. *pelargonii* (*Xhp*) from different suppliers in 2020.

Sample/batch	Date	Supplier	Cv	N sub-samples	per sub-sample	Total	% inf
S2352	07/04/20	1	A	5	40	200	<1.5
S2359	24/04/20	1	B	6	40	240	<1.3
S2370	15/05/20	1	B	6	40	240	<1.3
S2389	29/05/20	2	C	6	40	240	<1.3
S2398	08/07/20	1	B	6	40	240	<1.3
S2397	08/07/20	3	D, E	6	40	240	<1.3

In 2021 the sampling plan was modified to include more batches than in 2020. A total of 70 sub-samples, representing 18 batches of plug plants were tested/examined. The results of dilution plating are summarised in Table 12. In two cases (S2769 and S2803), visible symptoms were observed on almost every plant, in these cases direct isolations were done to confirm they were caused by *Xhp*, instead of the leaf wash performed on samples of symptomless leaves. In one case was the pathogen detected by leaf wash plating (S2886). The values provided for % infestation in the table are the maximum likelihood estimates based on the numbers of positive/negative sub-samples, or the upper 95% confidence limit when all sub-samples were negative.

In 2021, in addition to dilution plating on selective media, we also tested most samples by bioPCR. The bioPCR results were given a score according to the density of the band observed in the gels: 0 for no band, 2 for a very strong/overloaded band, 1 for a clear band, 0.5 for a faint but definitely present band, 0.1 for very faint band. In many cases we obtained positive bioPCR results when the plating tests were negative. In the one case where we had a positive detection by plating (S2886) we also obtained a high bioPCR score, but in most other cases the bioPCR score was lower (0.5 or 1).

Initially we considered that many of these results could be false-positives. We therefore repeated the PCR reactions under more stringent conditions (by increasing the annealing temperature), and only results giving a score of 0.5 or more with the higher annealing temperature were considered positive.

Table 12. Summary of tests on batches of Geranium plug plants for *Xanthomonas hortorum* pv. *pelargonii* (*Xhp*) from different suppliers in 2021. The shaded cells highlight the bioPCR positive samples.

Sample/batch	Date	Supplier	Cv	No. sub-samples	Total	Plating		BioPCR	
						% inf[1]	CFU/plant[2]	Score[3]	% inf[1]
S2611	09/04/21	1	K	4	110	<2.7	0	1 (1/3)	1.4
S2612	09/04/21	1	L	6	190	<1.6	0	0.5 (2/5)	0.5
S2613	09/04/21	1	A	6	150	<2.0	0	0	<2.3
S2614	09/04/21	1	G	3	60	<5.0	0	0	<5.0
S2615	09/04/21	1	B	6	190	<1.6	0	0	<1.6
S2624	28/04/21	1	B	4	110	<2.7	0	2 (2/4)	3.2
S2630	12/05/21	1	K	4	110	<2.7	0	1 (3/4)	6.4
S2636	27/05/21	1	B	4	110	<2.7	0	1 (2/4)	2.5
S2637	27/05/21	1	A	4	110	<2.7	0	2 (3/4)	6.4
S2691	07/07/21	1	B	4	110	<2.7	0	2 (4/4)	>2.7
S2713	15/07/21	4	M	3	70	<4.3	0	0.5 (1/3)	1.6
S2755	23/07/21	3	D	2	44	<6.8	0	0.5 (2/2)	>0.6
S2756	23/07/21	3	E	1	24	<13	0	0	<13
S2757	23/07/21	3	P	1	4	<75	0	0.5	>1.3
S2769	09/08/21	5	V	1	100	85*	>1E6	nt	nt
S2801	09/09/21	1	B	1	40	<7.5	0	1	>0.13
S2802	09/09/21	4	Q	2	60	<5.0	0	0.5 (1/2)	2.3
S2803	09/09/21	1	N	1	104	100*	>1E6	nt	nt
S2886	19/10/21	5	V	4	96	1.2	1.1E+04	2 (1/4)	1.2

Notes:
[1] Maximum likelihood estimate base on the proportion of positive/negative sub-samples.
[2] Maximum numbers of *Xhp* detected per plant.
[3] Maximum PCR (band intensity) score (see text for details), and the proportion of positive sub-samples in parentheses.
* % Inf based on visible symptoms.

In the spiked samples a positive plating result was obtained for 12 out of 13 samples. In most cases the number detected was slightly less than the expected number. The sample in which *Xhp* was not detected by plating had a much higher ratio (22.3) of background numbers to the target numbers compared to the other samples (max 3.1). Positive bioPCR

results were obtained for all of the samples tested, including the spiked sample that was negative in plating (S2624/4sp D2).

Table 13. Detection of *Xanthomonas hortorum* pv. *pelargonii* (*Xhp*) in spiked geranium leaf wash samples, by plating and bioPCR. The sample codes indicate the original sample/sub-sample number and 'sp' to indicated that it is a spiked sample. D0,D1, D2 indicate different dilutions of the inoculum used to spike the sample.

Sample	Plating (CFU/mL)			BioPCR	Ratio O:E[4]
	Others[1]	Expected *Xhp*[2]	Actual *Xhp*[3]		
S2611/4sp	2.1E+06	3.1E+06	1.4E+06	2	0.67
S2612/6sp	3.3E+04	3.1E+06	3.4E+06	2	0.01
S2613/6sp	2.1E+06	3.1E+06	1.1E+06	2	0.67
S2614/3sp	6.6E+06	3.1E+06	2.7E+05	2	2.14
S2615/6sp	1.8E+04	3.1E+06	3.0E+06	2	0.01
S2624/4sp D1	8.7E+04	1.0E+05	8.7E+04	2	0.83
S2624/4sp D2	1.2E+05	5.5E+03	0	1	22.3
S2630/4sp D0	3.4E+05	3.4E+05	2.0E+05	2	1.00
S2630/4sp D1	2.5E+04	1.8E+04	1.4E+04	2	1.40
S2636/4sp	7.9E+04	3.6E+04	2.5E+04	2	2.19
S2637/4sp	1.1E+05	3.6E+04	1.4E+04	2	3.10
S2691/3sp	2.6E+05	9.1E+04	1.4E+05	nt	2.88
S2756/1sp	2.9E+04	1.9E+06	1.1E+06	2	0.02

[1] No. of 'other' (not *Xhp*) bacteria, based on counts on BCBC medium,
[2] Expected no. of *Xhp*, based on counts on the inoculum used to spike the sample.
[3] Actual no. of *Xhp* detected.
[4] Ratio of Others to Expected.

Disease in production

In 2020 only a single assessment of production plants was made, in September; visible disease symptoms were observed in some of the plant material derived from tested plug plants, with disease incidence up to 100%.

In 2021 around 30 batches were observed on up to four occasions during the season. All disease observations were confirmed by isolation and the identity of the isolates further confirmed by PCR on at least one occasion for each batch. There was a marked difference in disease levels between the two sites visited. At Site 1, with no recent history of Geranium production, Xanthomonas leaf spots were never observed and plants remained apparently disease-free throughout the season. At Site 2 a complicated picture emerged:

- Batches of overwintered plants, recorded as infected in the previous year, displayed symptoms in early April and often showed 100% incidence by late May.
- Batches of plants derived from current season's plug plants initially appeared healthy soon after potting in late May, but showed 100% incidence by late July. Many of these were initially set out on the same bed as an overwintered batch that was known to be infected in the previous year.
- Batches of plants from current season's plug plants that initially appeared healthy but were sold quickly so that no further observations could be made. These were often set out on beds well away from over-wintered material.

We attempted to fit logistic models to the disease incidence data in order to estimate r-values (apparent infection rate) (Table 14). It should be noted that due to the rapid increase in disease between assessment visits in May and July and the concomitant lack of intermediate data points during this rapid increase, the estimates cannot be considered very reliable. When fitting the models, time zero was set at the date of potting, thus values for batches potted in in the previous year are generally lower.

Table 14. Estimated r-values (r, apparent infection rate) for Xanthomonas leaf spot in different batches and cultivars of hardy geraniums at one nursery in 2021. Values were obtained by fitting a logistic disease progress model to disease incidence.

Batch	Cv	Potted	r
013801	A	15/07/20	0.17
013805	B	15/07/20	0.11
116071	H	04/09/20	0.06
128568	K	04/05/21	0.17
128580	T	04/05/21	0.22
129332	A	04/05/21	0.20
130567	B	05/05/21	0.12
130808	G	30/04/21	0.22

Spread experiment

No symptoms were observed and the pathogen was not detected prior to the introduction of the inoculated plants. The inoculated plants were placed in the centre of each block on 19 July 2021.

For the block of 64 plants grown under protection with sub-irrigation, no disease symptoms were observed at any assessment, other than on the original inoculated plant. At the end of the experiment the pathogen (*Xhp*) was not detected in leaf washes of symptomless leaves collected from 28 plants. Except for the inoculated plant, these plants were retained and moved to a different location and grown on in the open over the winter and following year

(2022). Plants were carefully inspected on several occasions in the following spring and summer: no symptoms have been seen.

For the block of 121 plants grown in the open with overhead sprinkler irrigation, disease symptoms were observed on increasing numbers of plants as the experiment progressed. The initial inoculated plant represented an initial infection level of 0.8%, and at the final assessment symptoms were observed on 21.4% of plants. At the end of the experiment, the pathogen was also detected in all leaf washes of symptomless leaves collected from 22 plants. A few plants died during the course of experiment, but we were not able to ascertain if this was a result of infection with *Xhp*.

The final disease map at the completion of the experiment is shown in Fig 4. A graph showing the overall development of disease symptoms over time is shown in Fig. 5. Fitting a logistic model to the disease incidence data gave an estimated r-value (apparent infection rate) of 0.031 per day.

This r-value was then used to estimate the health standard required in plug plants to limit disease incidence to 10% over a six month growing period (i.e. for plants potted 01-April and grown on until 30-Sept)

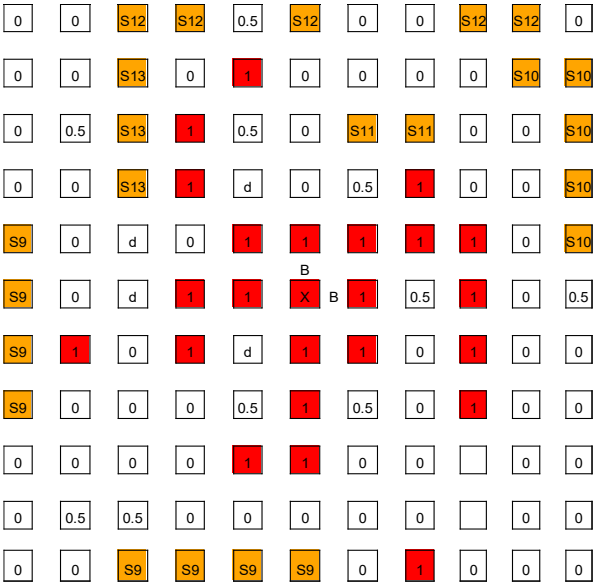


Figure 4: Map of Geranium plants with symptoms of Xanthomonas leaf spot on 11-Nov-2021. Red boxes indicate plants with symptoms. Orange boxes represent symptomless plants sampled on 11-Nov-2021 and on which *Xhh* was detected. The inoculated plant is in the centre and marked with an 'X'. Pots with a value of 0.5 are suspected of being infected, 'd' indicates plants that have died.

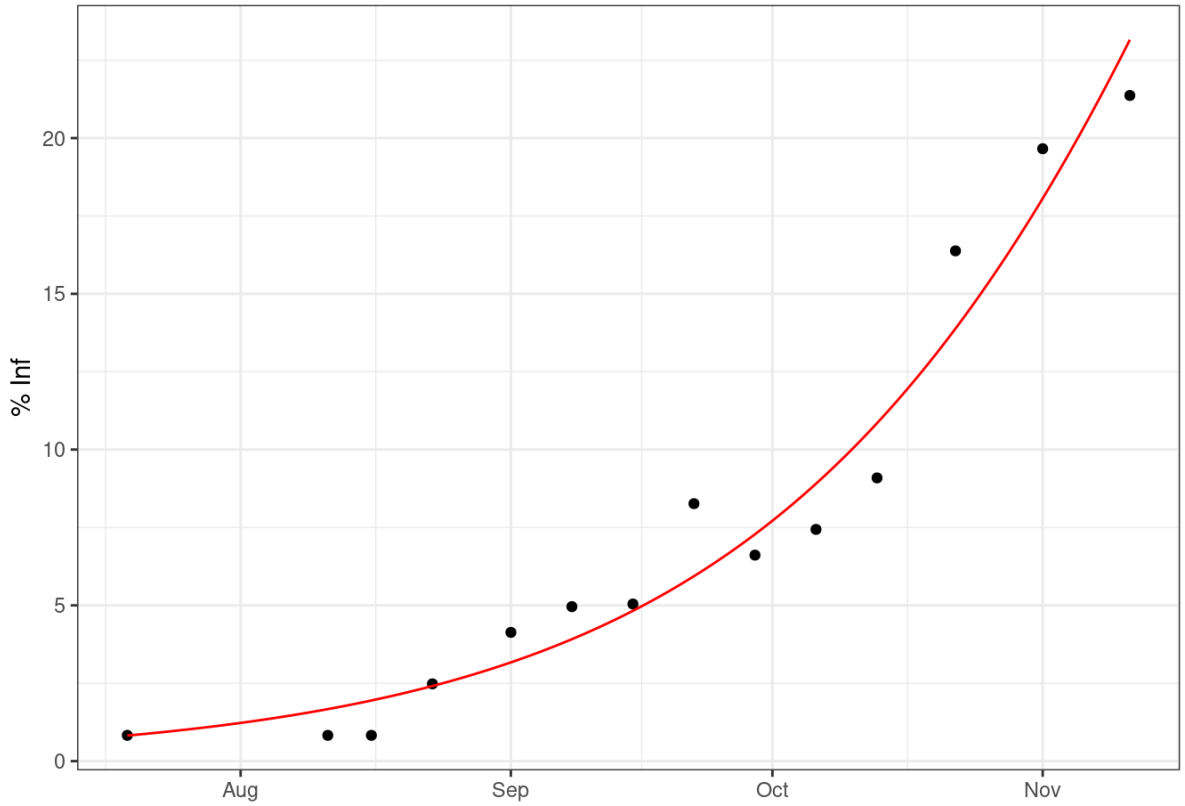


Figure 5. Disease progress in a block of hardy geranium plants growing outdoors with overhead irrigation, following inoculation of a single plant in the centre of the block. The fitted line in red is derived from a logistic model with an r-value (apparent infection rate) of 0.031 per day.

Discussion

In 2020, observations at the case-study nursery provided very strong circumstantial evidence that some batches of plug plants were infested with *Xhp*, but the pathogen was never detected in the plug plants tested at delivery to the nursery. As we did not test every batch, it is possible that we failed to test the infested batches; alternatively it could be that the level of infestation in the tested batches was below the detection limit of the test (i.e. <1.3%), and that there was significant secondary spread on the nursery. However, it had also been noted that there were high background numbers of 'other' bacteria on many of the test plates: these can mask the presence of the target pathogen, thereby effectively reducing the analytical sensitivity of the test. Therefore, in 2021, we modified our approach to test more batches of plug plants, combined with additional bioPCR testing, and testing of spiked samples.

In 2021, we detected *Xhp* in three batches of plug plants on delivery to the nursery. Two of these were detected due the presence of high levels of leaf symptoms that were then confirmed as *Xhp* by direct isolation from the lesions. In the third case the pathogen was detected by dilution plating of a leaf wash of symptomless leaves. In this sample, numbers of *Xhp* were similar to those of the background population. However, we again failed to detect *Xhp* by leaf-wash plating in the majority of batches of plug plants, but again noted high background numbers of 'other' bacteria in many samples.

In the majority of spiked samples, where relatively high numbers of *Xhp* were added compared to the background numbers, we were able to detect the pathogen by conventional plating. In the one case where background numbers were more than ten times the pathogen population we failed to detect *Xhp* by conventional plating. Thus confirming our earlier suspicions that false-negatives were possible when our ability to detect relatively low numbers of the pathogen was hampered by the presence of high background numbers of other bacteria on the dilution plates.

BioPCR testing indicated that a significant proportion of batches that were negative by conventional plating, may be infested with *Xhp*. We were initially sceptical of these results and considered that some or all could be false-positives. A common concern with PCR testing is that it may detect residual DNA from dead cells; this is obviated in bioPCR, as it relies on some growth (even if invisible) of the target pathogen. Another concern was that the results were due to non-specific reactions, we therefore re-tested all initially positive samples under more stringent PCR reaction conditions (a higher annealing temperature to reduce the likelihood of detecting mismatched sequences).

BioPCR testing of the spiked samples gave positive results for all samples, including the sample with relatively lower numbers of *Xhp* added and where we failed to detect the pathogen by conventional plating. This indicates that the sensitivity of bioPCR may be greater than conventional plating, especially when the numbers of background bacteria are relatively high.

Taken together, even if some of the (weaker) bioPCR results are false-positives, it is likely that those samples where we obtained a strong positive signal (score 2) are genuine true-positives, and that in these cases the numbers of *Xhp* may have been relatively lower than the background populations, resulting in detection failure by conventional plating. These results strongly suggest that our suspicions in the first year were correct: that some of the negative plating results may be false-negatives. Further work is needed to quantify the sensitivity and impact of background populations on detection both by conventional plating and bioPCR.

The lack of sensitivity for conventional plating was due to a relative lack of selectivity of the media in the presence of apparently high numbers of other bacteria in the plug plant samples. Further work is needed to improve these media or develop alternatives.

As well as being more sensitive than conventional plating, the bioPCR has potential to provide results in a much shorter time-frame; i.e. within three days of initial sample processing, rather than the five to seven for dilution plating with PCR confirmation. This could allow growers to have samples of plugs tested upon receipt and only accept/pot on if the BioPCR result is negative. Potentially direct PCR on the leaf wash extracts could further reduce the time-frame for a result, but is likely to be less sensitive. Further work would be needed to further validate and refine the bioPCR method for routine application.

The major difference in disease levels (complete absence at one site) in production from the split batches of plugs grown on at two different nursery sites suggests that local spread of the pathogen amongst different batches and particularly over-wintered batches plays an important role in the development of disease. Due to the relatively high levels of potential inoculum in over-wintered production, we cannot be certain of the relative contribution of the possible low levels detected by bioPCR in some batches of plug plants. However, the first disease seen in late May in the 2021 production (4% incidence) was in a batch of plants derived from plug plants with a positive bioPCR result (that indicated 1.4% incidence in the plug plants).

The very rapid increase in disease incidence on the production nursery during June 2020, was unexpected, and so we did not obtain data at this critical time during epidemic development. The estimated r-values of 0.17 to 0.22 are particularly high, especially when compared to the value of 0.03 obtained in the spread experiment. Temperatures in June 2021 were relatively high (implying frequent irrigation) and there were a couple of significant rainfall events. However, we suspect that there may be other factors driving the rapid epidemic development. Possibly this is an indication of widespread low level asymptomatic contamination of plants (that is already present on the plug plants), so that we are observing a local within-plant increase in pathogen populations on individual plants rather than plant-to-plant spread. When visiting in July we noted that one of the heavily infected batches was flowering profusely with much bee activity. We took a small sample of just seven flowers from this batch and processed them in a similar way to the leaf wash samples, and were surprised to find a mean population of 1.1 x 10^5 CFU *Xhp* per flower. We therefore speculate that that bees and other pollinating insects may also play a role in the apparent rapid spread of the pathogen.

The spread experiment clearly demonstrated the critical role of overhead water (from rain or irrigation) in the spread of geranium bacterial leaf spot. In the plants grown outdoors with overhead irrigation, the pathogen had effectively spread to all plants by the end of the experiment. However, the estimated r-value of 0.03 for disease symptoms was considerably smaller than the values obtained on the production nursery (0.17 to 0.2). This may simply be a reflection of the different weather conditions and time of year, but we also speculate that perhaps the primary inoculum pressures were quite different, for example high levels of disease (and inoculum) on over-wintered infected plants at the beginning of the growing season.

Based on the r-value of 0.031 from the spread experiment a health standard of <0.03% would need to be achieved in plug plants to ensure disease incidence did not exceed 10% by the end of the growing season. To achieve this standard would require testing of around 9,000 plants to achieve 95% probability of detection with a test method that is 100% "reliable". Corrections for small batch sizes would still mean that in a batch of 1,000 plants 900 plants would still need to be tested. Clearly such a testing or quality assurance programme would be done most effectively by the plug-plant producers, with further efficiencies gained by focussing on the health status of the parental material. It would potentially add to the cost of plug-plant production (and the prices) but the reduction in losses of final 3 or 5 litre pots will likely outweigh such additional costs. Note that if we were to make the same calculation using the r-values from the nursery (e.g. 0.17), the required health standard becomes unfeasibly small (10^{-13}).

The complete absence of any detectable spread of the pathogen and subsequent freedom from disease in the following year in the plants grown under protection with sub-irrigation, as well as providing evidence for the critical role of overhead rain/irrigation in the spread of disease, also demonstrates a potential system for production and maintenance of healthy parental material. Furthermore, a similar system could also be used for the production of plug plants as demonstrated for the high-health brassica transplants. This then in turn would greatly reduce the burden of testing.

Conclusions/Recommendations

- *Xhp* may be present on plug plants in the absence of symptoms.
- Growers should press suppliers for assurances of the health status of plug-plants.
- Growers should avoid/reduce overhead irrigation as much as possible.
- It is essential that growers should not mix batches of over-wintered and current season's production on the same beds.

- Growers should grow different batches as far apart as possible.
- Discard (remove and destroy) infected plant as soon as possible – do not overwinter material that is known to infected.
- Propagators should regularly test parental material for the presence of *Xhp*, and discard any that are positive.
- Propagators should maintain parental material under protection with sub-irrigation.

Hedera and Xanthomonas leaf spot

Bacterial leaf spot of ivy is caused by *Xanthomonas hortorum* pv. *hederae* (*Xhh*). This disease has been an on-going issue for many years, resulting in growers ceasing production of ivies. Work was focussed on two areas: detection of the pathogen on bought-in plant material and determining the rate of spread.

Materials and methods

Detection in bought-in plants

Samples of leaves were collected from liner plants at the time of delivery to the nursery whilst still in the Dutch trolleys. Samples consisting of one or more sub-samples of up to 30 leaves were collected from each batch and taken to the laboratory for testing. Sub-samples were stomached in sterile saline plus tween 20, diluted and plated on two selective media BCBC and modified Tween (mTW) (Holcroft & Roberts, 2002). Suspect colonies were sub-cultured to sectored plates of YDC and confirmed as *Xanthomonas* by PCR with Xanthomonas-specific primers. The identity of isolates from each positive sample was also confirmed by testing for pathogenicity on ivy.

Spread experiment

A batch of ivy plants was propagated from parental plants with no previous history of disease. Leaf samples were also tested for the presence of *Xhh* prior to the start of the experiment. Plants were potted into 9 cm square pots of Levington CNS growing medium plus CRF and set out in two square blocks. One block (14 x 15 = 210) plants) was set out outdoors with an overhead irrigation system and the other block (8 x 8 = 64 plants) was set out in a polytunnel with a sub-irrigation system similar to that used for the high health brassica transplants. Two plants were inoculated with *Xhp* and nine days later one was placed in the centre of each block. The development of disease was then monitored at regular intervals and the location of diseased plants recorded on a map. At the final recording and end of the experiment, samples of symptomless leaves were collected from both outdoors and under protection. These were then transported to the laboratory and tested as above.

Data Analysis

The percentage of symptomless leaves infested with *Xhh* was estimated using maximum likelihood methods and STPro (Ridout & Roberts, 1995).

Data from the spread experiment were analysed using R (R Core Team, 2021). The disease maps were used to generate Cartesian coordinates (x,y) for each plant and its health status at each assessment date. Models were then fitted to the data using generalised linear modelling procedures (glm, binomial, logit link function) or non-linear modelling procedures (Levenberg-Marquardt type fitting algorithm).

Results

Detection on bought-in plants

When collecting leaves, visible symptoms were present in four of the five batches of liners examined at the point of delivery to the nursery. We therefore counted the numbers of plants with symptoms in a number of trays from each batch and made direct isolations from these leaves to confirm that they were caused by *Xhh*. We also collected symptomless leaves from each batch and performed a leaf wash as originally planned. In addition we also did a leaf wash on a single infected leaf. Results are summarised in Table 15. Where all sub-samples were either negative or positive, the value for the % infection (% Inf) is the upper or lower 95% confidence limit of the estimate.

Table 15. Summary of tests on ivy liners at the point of delivery to the grower. The % Inf is the estimated infestation level in the symptomless leaves. The symptoms is the % of plants with visible symptoms.

Sample	Date	Cv	No. of sub-samples	Total	% Inf	Max CFU/leaf	Symptoms (%)
S2631	19/05/21	Gloire de Merengo	3	75	1.6	8.4E+05	1.0
S2632	19/05/21	Dentata Variegata	3	75	<3.9	0.0E+00	<0.4
S2633	19/05/21	Glacier	2	60	>0.84	1.4E+06	75.9
S2634	19/05/21	Goldheart	2	60	>0.84	4.4E+06	66.7
S2635	19/05/21	Goldchild	2	60	>0.84	2.7E+06	91.7
S2633	19/05/21	Glacier	Single infected leaf			4.6E+07	

Spread experiment

No symptoms were observed and the pathogen was not detected prior to the introduction of the inoculated plants. The inoculated plants were placed in the centre of each block on 12 July 2021.

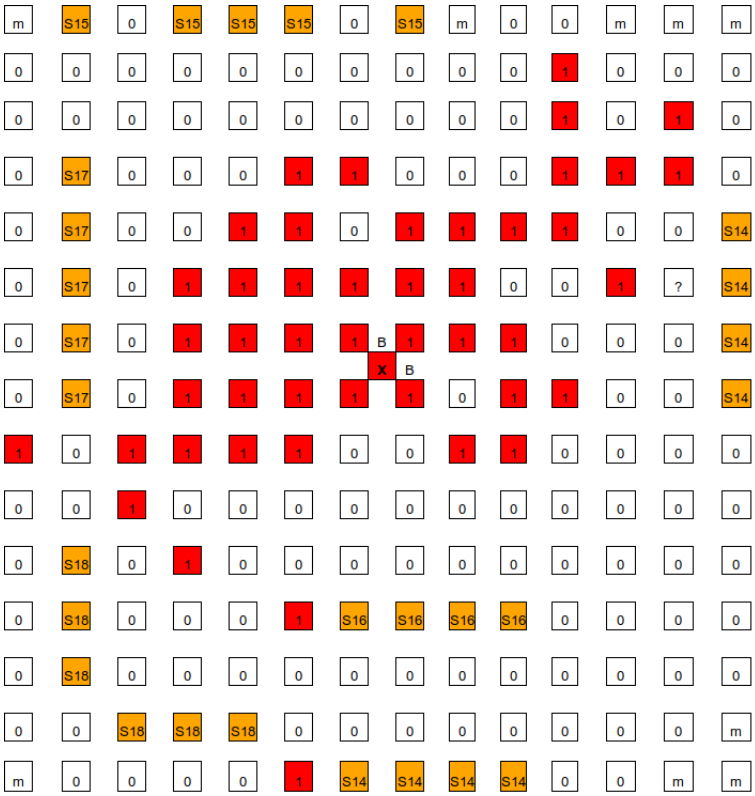


Figure 6. Map of ivy plants with symptoms of Xanthomonas leaf spot as at 11-Nov-2021. Red boxes indicate plants with symptoms. Orange boxes represent symptomless plants sampled on 11-Nov-2021 and on which *Xhh* was detected. The inoculated plant is in the centre and marked with an 'X', 'm' indicates missing plants.

For the block of 120 plants grown under protection with sub-irrigation, no disease symptoms were observed at any assessment, other than on the original inoculated plant. At the end of the experiment the pathogen (*Xhh*) was not detected in leaf washes of symptomless leaves collected from 32 plants.

For the block of 200 plants grown in the open with overhead irrigation, disease symptoms were observed on increasing numbers of plants as the experiment progressed. The initial inoculated plant represented an initial infection level of 0.5%, and at the final assessment symptoms were observed on 23.3% of plants. At the end of the experiment, the pathogen was also detected in all leaf washes of symptomless leaves collected from 20 plants.

The final disease map at the completion of the experiment is shown in Fig 6. A graph showing the overall development of disease over time is shown in Fig. 7. Fitting a logistic model to the disease incidence data gave an estimated r-value (apparent infection rate) of 0.036 per day.

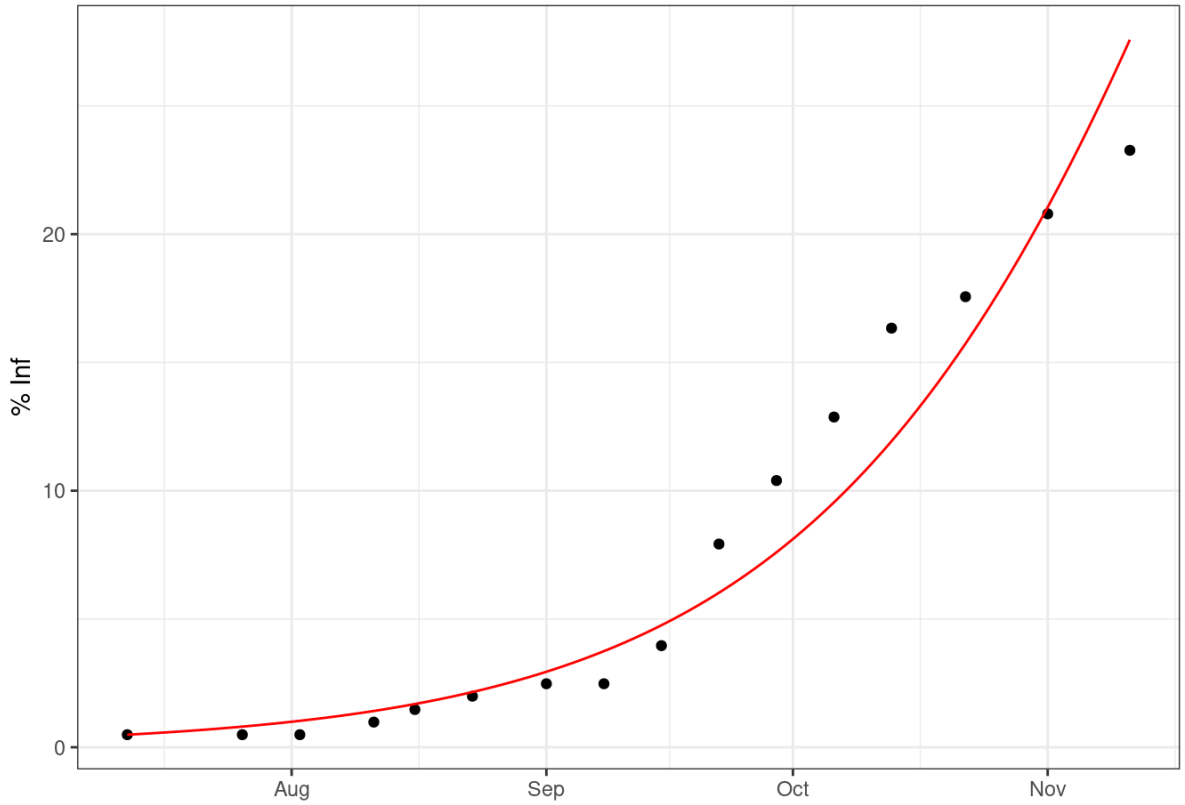


Figure 7. Disease progress in a block of ivy plants growing outdoors with overhead irrigation, following inoculation of a single plant in the centre of the block. The fitted line in red is derived from a logistic model with an r-value (apparent infection rate) of 0.036 per day.

Discussion

The presence high levels of symptoms on the liners at the point of delivery provided conclusive evidence that they are the primary source of infection in production. These plants also had relatively high numbers of the pathogen (~10^6 CFU/leaf) even on symptomless leaves. One variety 'Dentata variegata' appeared to be free from the pathogen: we did not observe any leaf spot symptoms and we did not detect the pathogen on symptomless leaves. It would be tempting to consider that this variety might be resistant, but previous work (Holcroft and Roberts 2002) indicates that it is fully susceptible. It seems more likely that the reduced levels are a result of the health status of the mother plants, and gives a clear indication that it is possible to produce liners with low or even zero levels of infestation.

In the spread experiment, we did not observe any spread of disease symptoms or detect any symptomless spread of *Xhh* on plants grown under protection and watered via a sub-irrigation system. In contrast we observed significant spread of disease symptoms and detected symptomless spread of the pathogen to the edge of the block on plants grown

outdoors and watered via an overhead sprinkler system. The percentage of plants of plants with symptoms increased from an initial level of 0.5% to a final level of 23% over the four month duration of the experiment. Thus the spread experiment has demonstrated the importance of rain/overhead irrigation in plant-to-plant spread of the pathogen and subsequent disease development.

Given that we detected the pathogen on all samples from symptomless plants at the end of the experiment it seems feasible that all plants in the block could potentially be carrying the pathogen irrespective of symptoms. Thus the absence of symptoms cannot be taken as a reliable indicator of the health status of batch of plants. Conversely symptoms found on any plant in a batch should be taken as a indicator that all plants in the batch could be carrying the pathogen and could subsequently develop symptoms.

Based on the r-value of 0.036 from the spread experiment a health standard of <0.013% would need to be achieved in liners to ensure disease incidence did not exceed 10% by the end of the growing season. To achieve this standard would require testing of around 23,000 plants to achieve 95% probability of detection. Corrections for small batch sizes would still mean that in a batch of 1,000 plants 959 plants would still need to be tested. Clearly such a testing or quality assurance programme would be done most effectively by the liner producers, with further efficiencies gained by focussing on the health status of the parental material. It would potentially add to the cost of plug-plant production (and the prices) but the reduction in losses of final 3 or 5 litre pots will likely outweigh such additional costs.

Necessarily for the spread experiment, it was essential that we were able to source and produce disease and pathogen-free plants. These plants were propagated from mother-plants obtained from sources with no previous history of disease, which were then grown-on under protection and observed closely, and finally tested to confirm their health status. Their continued freedom from disease symptoms or detectable levels of the pathogen when grown under conditions that limited the potential for pathogen spread clearly demonstrates that production of disease-free ivies is feasible when careful attention is given to the health status of the parental material. Thus even though there may be limited or no options for chemical control, it is feasible to manage the disease through the use of healthy propagation material.

We suspect that widespread issues with *Xhh* in ivies are the result of failure of propagators to monitor the health status of the mother plants. This was borne out by the remarkably high levels of visible disease symptoms apparent in liners supplied to a production nursery.

Conclusions/Recommendations

- Bacterial leaf spot of ivies on production nurseries is most likely the result of using infested planting material (liners).
- *Xhh* may be present on plants in the absence of symptoms.
- Production of ivies free from bacterial leaf spot is feasible.
- Growers should carefully inspect bought-in plants upon receipt and reject any batches with symptoms of bacterial leaf spot.
- Growers should seek assurances from propagators/suppliers that bought-in plants/liners are pathogen-free.
- Growers should avoid/reduce overhead irrigation as much as possible.
- Propagators should monitor and test parental material for the presence of *Xhp*, and discard any that are positive.
- Propagators should maintain parental material under protection with sub-irrigation.
- Propagators should only take cuttings from plants which are known to be free from *Xhh*.

Delphiniums and bacterial blotch

Bacterial blotch of Delphiniums is caused by *Pseudomonas syringae* pv. delphinii. The disease had been seen in some batches of plants on the case-study nursery in previous years, therefore initial work focussed on sampling and testing plug plants as they arrived on the nursery.

Materials and methods

Following an initial nursery visit, a sampling scheme was devised for collection of samples from plug plants at the time of delivery to the nursery and based on the expected numbers of plants in each batch. Samples, consisting of six or seven sub-samples of 40 leaves, were collected by the nursery from each batch and sent to PHS for testing. Most batches consisted of multiple varieties delivered by a supplier on the same date, with each variety represented by one or more sub-samples. Sub-samples were stomached, diluted and plated as described in HNS 178 (Roberts, 2013b)

A follow-up visit was made to the nursery and production resulting from the plug plants was thoroughly inspected for the presence of disease symptoms.

Results

Psd was not detected in any of the plug plants (see Table 16). The values for infection in the table represent the upper 95% confidence limit. The grower did not report any disease during the season, and no disease was detected during a follow-up visit and inspection of material.

Table 16. Summary of tests on Delphinium plug plants for the presence of *Pseudomonas syringae* pv. *delphinii*.

Sample/lot	Date	Supplier	N sub-samples	N per sub	Total	% Inf
S2354	20/04/20	4	3	40	120	<2.5
S2355	20/04/20	5	3	40	120	<2.5
S2356	20/04/20	6	5	40	200	<1.5
S2357	20/04/20	1	5	40	200	<1.5
S2363	24/04/20	2	1	40	40	<7.7
S2360	24/04/20	4	3	40	120	<2.5
S2361	24/04/20	5	6	40	240	<1.3
S2362	24/04/20	4	1	40	40	<7.7
S2363	24/04/20	2	1	40	40	<7.7
S2371	15/05/20	1	1	40	40	<7.7
S2372	15/05/20	1	7	40	280	<1.1
S2390	29/05/20	6	5	40	200	<1.5
S2486,7	10/09/20	6	2	48	96	<1.0
S2489	10/09/20	6	1	40	40	<7.7

Discussion

The absence of any apparent disease during production is consistent with the apparent absence of the pathogen, *Psd,* in any batches of plug plants. This is in marked contrast to the experience with hardy Geraniums. It may be a result of differing biology and interactions of the pathogens with the environment given that they belong to different bacterial genera. Alternatively it may be due to different predominant propagation systems used for the two host genera: the majority of the delphiniums plug plants are produced from seed, whereas the geraniums are produced from micro-propagated cuttings.

Given the absence of any disease observed in 2020, a decision was made to focus efforts on geraniums in 2021. Nevertheless, observation of production material in 2021 continued to indicate freedom from bacterial leaf spot.

High Health Transplants

Previous work demonstrated that even when a bacterial pathogen is present, production of transplants and cuttings, using a sub-irrigation system (capillary matting/ebb-flood) rather than overhead can give control equivalent to that achieved with repeated sprays with copper oxychloride. Brassica transplant production was used as a model system, but we believe that a similar approach can subsequently be applied to other crops (e.g. cucurbits, protected ornamental plug plants and similar) that could be grown under such a system.

Material and methods

The system was set up at one end of a bay in a commercial brassica transplant production nursery.

In 2020, wooden pallets were placed on the floor of the glasshouse to provide a slightly raised platform and enable rapid drainage. Polystyrene sheets were then laid on top of the pallets to provide a flat surface, and the whole lot levelled by placing wedges under the pallets as necessary. A raised edge was created around the perimeter of the area by taping ~2 cm wide strips of 7.5 mm foam insulation around the edges. The polystyrene sheets were then covered with a layer of thick black polythene, followed by a layer of capillary matting and a top layer of Tex-R fabric. This fabric is coated with SpinOut®, a copper-based compound that inhibits rooting into the matting. The matting and fabric were allowed to overlap the edge at one end with sufficient excess to reach the floor. Trickle tape was then laid across the length of the area spaced 40 cm apart (width of module tray) and connected to a header pipe. The header pipe was in turn connected to the glasshouse irrigation system via a filter and pressure reducing valve.

Fifteen '345' module trays were filled with growing medium and sown according to normal practice at the nursery. The trays were placed on the bed, and the irrigation valve opened to irrigate via the trickle tape and thoroughly wet the matting until excess was beginning to drain off the bed. In 2020, a light overhead sprinkling of water was also applied by hand to settle the growing medium in the module cells.

The plant raiser was given little specific instruction and requested to open the irrigation valve as he saw fit to ensure normal growth, with the duration of each irrigation cycle sufficient for water to puddle when the matting was depressed.

In 2021, a larger number of trays (40) were produced. To accommodate the larger scale some modifications were made. Instead of wooden pallets, up-turned 5" pots were laid out on the floor of the glasshouse. Polystyrene sheets were then laid on top of the pots to

provide a flat surface, and the whole lot levelled up as much as possible by using multiple pots in low spots. Other aspects were then as in 2020.

In both years, plants were delivered to a commercial grower and planted alongside normal production.

Results

In both years plants grew normally and produced transplants which were indistinguishable from conventionally produced transplants. The matting used in 2020 was re-used in 2021. In 2021, there was no discernable difference between plants growing on the re-used matting and new matting.

The plant raiser reported that they were satisfied with the quality of the plants produced and had no particular issues, apart from uneven growth in some cells of two trays that straddled uneven sections of polystyrene sheet, this required turning of the trays. In 2021, a low level of downy mildew was observed at 19 days after sowing and a single spray applied. Overall, the plant raiser reported that the frequency of watering was reduced compared to conventional overhead watered plants, raised simultaneously in the same house, and that less feeding was also required.

At six weeks after sowing the plants were delivered to the grower and planted and were then monitored until harvest (see black rot section). The grower reported that they were satisfied with the quality of the plants delivered and had no particular issues. No issues were reported when planting and final yields exceeded those of equivalent conventionally produced plants.

Discussion

We have shown that it is possible to produce high-health brassica transplants in standard 345 module trays using a quasi ebb and flood sub-irrigation system. The system required less water, less feeding and fewer pesticide applications than conventionally produced transplants.

A priori, a key concern was that growing plants on capillary matting and without the benefits of air-pruning of roots would result in plants that suffered from root disease problems or had damaged roots and thus might fail to establish well in the field. This proved not to be the case, despite re-using the matting and covering fabric in the second year. The Tex-R fabric prevents rooting through into the matting and the root systems seem to be comparable with those of conventional transplants.

The use of high health transplants resulted in substantial reductions in disease in the field and yield increases in both years (see black rot section)

Conclusions

- The production of high-health transplants or plug plants using a sub-irrigation is feasible, with potential to reduce subsequent bacterial disease levels and increase yields in production.

Knowledge exchange

In 2020, at least 14 visits were made to growers, together with phone conversations and email exchanges of information.

In 2021, around 16 visits were made to growers during the year, together with phone conversations and email exchanges of information.

In 2022 at least four visits were made to growers, together with phone conversations and email exchanges of information.

The following formal presentations were made:

- Brassica Growers Association, 04-Feb-2020
- AHDB podcast, 13-Aug-2020
- Project review meeting, 16-Sep-2020
- Brassica Growers Association, 17-Nov-2020

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