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Project leader:	Dr S J Roberts, Plant Health Solutions Ltd
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Location of project:	Warwick, Cawood, various grower sites
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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.

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CONTENTS

GROWER SUMMARY

Headline

High-health planting material produced with sub-irrigation has shown a significant benefit in terms of both disease levels and crop yield.

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 is a collaborative project between Plant Health Solutions (PHS), Stockbridge Technology Centre (STC), Warwick Crop Centre (WCC) and growers and will primarily focus 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 will be determined, the benefits of clean starting material will be demonstrated, and epidemiological data obtained to set health standards for starting material. We will also examine the feasibility of novel methods to produce high-health planting material as a second-line defence, and examine the potential for resistance deployment where we think this may be feasible. This report covers the second year of the project.

Summary

Brassicas and Black Rot

- Further seed testing has identified additional seed lots infested with Xanthomonas *campestris* pv. *campestris* (Xcc)
- *Xcc* was detected in several batches of transplants.
- More than 30 crops/locations have been walked/examined and levels of black rot assessed. High levels of disease were associated with known infested seed lots or transplants,

- High-health transplants gave a benefit in terms of both disease levels and yield, despite being surrounded by an infected crop.

Broccoli spear rot

- Despite inoculation with pathogenic strains, no disease developed in the resistance screening trial.
- We were able to demonstrate both seed to seedling transmission and spread during plant-raising.

Coriander and parsley bacterial blight

- We were unable to isolate any specific bacterial pathogen from parsley samples.

Cherry laurel and bacterial shot-hole

- Cv Otto Luyken has been successfully established in tissue-culture, with reasonable multiplication rates. A batch of plants was transferred to a commercial tissue-culture company for further multiplication and were successfully weaned.

Hardy Geraniums and Xanthomonas leaf spot

- *Xanthomonas hortorum* pv. *pelargonii* (*Xhp*) was detected in several batches of plug plants.
- As well as introduction with propagation material, it would appear that spread of disease amongst batches of plants of different ages may be important for epidemic development on the nursery,
- Further investigation of the detection method, suggests that false-negatives may be an issue.
- A spread experiment is underway to provide data for use in setting health standards.

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.
- A spread experiment is underway to provide data for use in setting health standards.

Novel Production System

- The sub-irrigation system trialled last year was scaled-up.

- Transplants were again raised successfully and both plant-raiser and grower were happy with the quality of the plants.
- The trial system needed less watering and feeding than conventional production.

Financial Benefits

At the present time, no specific financial benefits have been identified.

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.

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 is a collaborative project between Plant Health Solutions (PHS), Stockbridge Technology Centre (STC), Warwick Crop Centre (WCC) and growers, and will primarily focus 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 will be determined, the benefits of clean starting material will be demonstrated, and epidemiological data obtained to set health standards for starting material. We will also examine the feasibility of novel methods to produce high-health planting material as a second-line defence, and examine the potential for resistance deployment where we think this may be feasible.

The primary aim of the project is 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. This report covers the second year of the project.

Brassicas and black rot

Black rot of brassicas is caused by *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. Fields containing brassica crops of interest were walked and the levels of (suspected) black rot disease incidence assessed. Where 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 requested from the seed companies and up to 60,000 seeds tested for each lot.

Transplant testing

A different approach to sampling and testing (compared to the first year) was followed, 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 methods for seed and transplant testing were as described in a previous project, FV 335 (Roberts, 2009) The seed test method was based on an International Seed Testing Association (ISTA) validated method (Roberts & Koenraadt, 2005) with the addition of a centrifugation step to improve 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 or mCS20ABN selective media. Suspect colonies of *Xcc* were then sub-cultured and their identity confirmed.

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. Suspect colonies of *Xcc* were then sub-cultured and their identity confirmed.

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

The transplants planted in year one were monitored at intervals over the winter along with the surrounding crop. Yield was assessed in three 'plots' in the centre of the high-health block and in three parallel plots in the surrounding crop. 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 included, 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.

Results

Seed testing

Seed testing was done on some additional seed lots (Table 1), and further testing is still on going for some lots of particular interest. Unfortunately, we were able to obtain any seed of some lots of interest, and only limited quantities of others thereby increasing the confidence limit of a negative test result (meaning that we can be less certain the 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*).

Sample (Lot)	Type	Cv	Source	N tested	N sub-samples	% inf
S2324	Kale	A	1	30,000	3	0.004
S2325	Kale	A	1	12,000	3	0.02
S2326	Kale	A	1	60,000	6	<0.005
S2327	Kale	A	1	60,000	6	<0.005
S2328	Kale	A	1	50,000	5	0.005
S2329	Kale	B	1	50,000	5	<0.006
S2330	Kale	B	1	50,000	5	0.009
S2375	Borecole	C	2	30,000	3	<0.01
Additions 2021:						
S2589	Broccoleto	G	5	15,000	3	0.008
S2590	Broccoli	H	6	10,000	2	<0.03
S2591	Cauliflower	J	2	2,000	2	<0.15
S2592	Cauliflower	K	7	4,000	2	<0.08
S2593	Cauliflower	L	4	5,800	2	<0.05
S2594	Cauliflower	M	4	4,500	2	<0.07

Transplant testing

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 four batches. Two 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 were 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 case less than reliable the 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 the previous year.

Table 2. Summary of tests on brassica transplants for *Xanthomonas campestris* pv. *campestris* (*Xcc*) at nursery 01. 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.

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

Table 3. 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.

Sample (Batch)	Type	Cv	Seed lot	Source	N tested	N_sub	% inf	CFU/plant
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	D		3	60	2	<5	
S2699	Kale	F		3	40	2	<8	
S2700	Broccoleto	G	S2589	5	20	1	<15	
S2701	Kale	Q		?	60	2	<5	
S2702	Kale	Y		9	20	1	<15	
S2703	Kale	S		4	30	1	<10	
S2704	Kale	V		?	60	2	<5	
S2705	Red Russian	R		10	24	2	<13	
S2706	Broccoletto	G	S2589	5	15	1	<20	
S2707	Savoy	N		1	20	2	<15	
S2759	Kale	A	S2609	1	56	2	2.4	3.2E+5
S2760	Kale	A	S2608-HW	1	88	3	<3	
S2761	Kale	A	S2609-HW	1	116	4	<2.6	
S2762	Kale	A	S2609	1	116	4	1	4.5E+4
S2763	Kale	B	S2330	1	120	4	1	5.7E+2
S2764	Kale	X		?	29	2	<10	

Fields assessments (2020-21)

More than 30 crops/locations were walked/examined and levels of black rot assessed on 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 borecole kale types
- water-soaked or pale papery lesions de-llimited by veins, particularly soon after planting

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. Crop N had the earliest and highest levels of infection in the field, the disease was also uniformly distributed in the field strongly indicating that the transplants were infested at planting.

The cruciferous weeds charlock and shepherd's purse were also present in in some fields. typical Xanthomonas lesions were seen on both and Xcc isolated. Initial results suggest that the strain(s) in weeds may not be the same as those in the surrounding crop.

Table 4. Summary of black rot disease levels (Max infection) and other details for some of the key crops followed over the Winter 2020-21.

Field	Type	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	N	32	100	nt	nt	Kale
MT	Brocoletto	G	32	75	nt	0.008	Kale
MT	Kale	A High Health	32	56	**	<0.005	Kale
MT	Kale	A	33	90	<2.1	0.005	Kale
MT	Curly kale	B	33	0.1	nt	0.009	Kale
CL	Kale	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	F	29	70	<1	nt	Rye/Vetch
CL	Brocoletto	G	29	100	nt	0.008	Rye/Vetch
CL	Kohlrabi	Near Broc	29	100	nt	nt	Rye/Vetch
CL	Kohlrabi	Far Broc	29	0	nt	nt	Rye/Vetch
KD	Kale	A	32	78	0.7	0.005	Kale
FL	Kale	F	29	0.1	<1	nt	Cereal
FL	Curly kale	O	29	0	<1.5	nt	Cereal

nt = not tested

High Health Transplants 2020-21

In the year 1 batch of 5000 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. The first infection observed was a lesion developing from the edge of a leaf that had been subject to bird (pigeon?) 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 (by 34%) yield for the high-health compared to the surrounding conventional crop (Table 5).

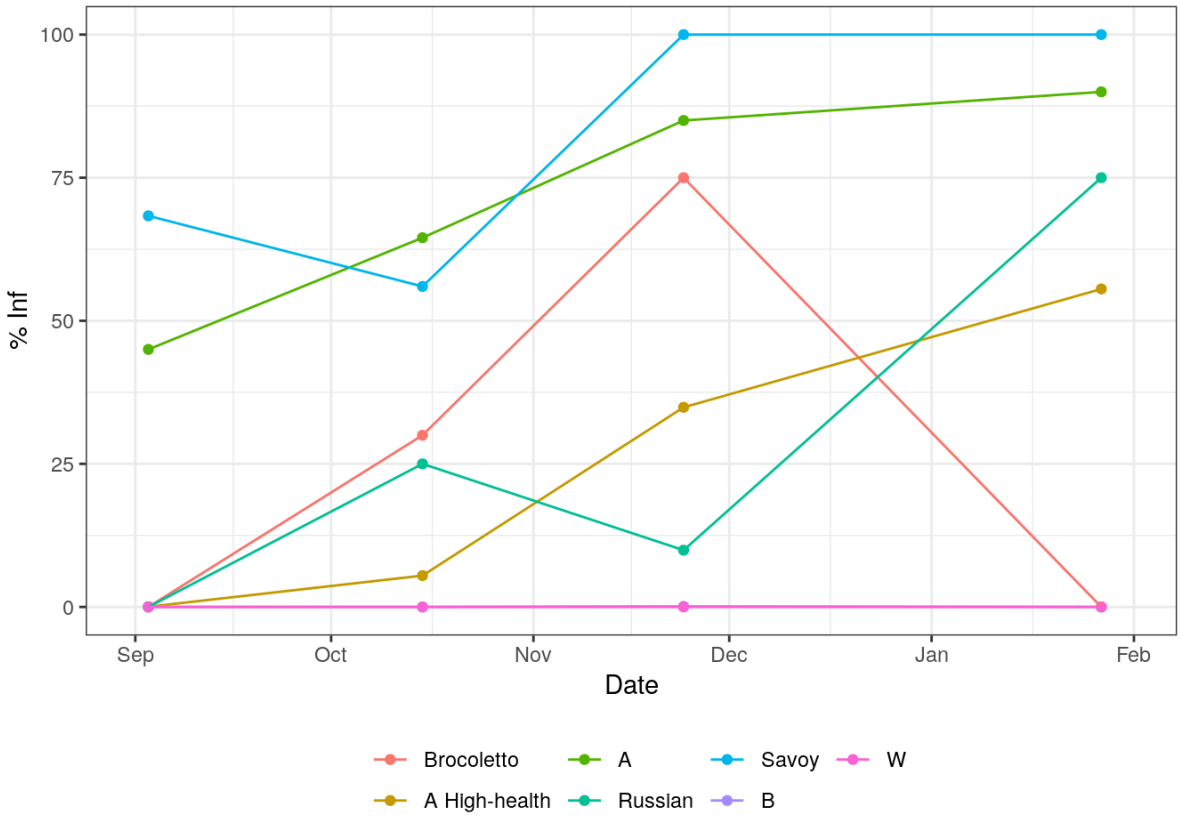

Figure 1. Development of black rot (disease incidence, % Inf) over time for several different brassica crops/varieties grown in the same field in 2020-21.

Table 5. Summary of yield measurements for kale crops grown from conventional and high-health transplants, 2020-21 season.

Transplants	Yield (t/ha)	Max. disease incidence
Conventional	5.5	90
High Health	7.4	56
s.e.	0.2	

High Health Transplants 2021-22

Transplants were successfully planted and will continue to be monitored over the winter until harvest.

Discussion

Seed tests have shown the presence of apparently low levels of *Xcc* in several seed lots. Further testing is underway and we will seek to obtain further seed lots where we have detected the pathogen in transplants.

Tests on transplants identified the presence of *Xcc* four batches in 2021, three were derived from seed lots which had been tested and given a positive test result. There were two batches (S2700 and S2706) derived from seed known to be infested that were negative, but given the limited numbers of plants tested resulting in a detection limit of ~15%, it is quite feasible that these batches were nevertheless infested at similar levels to those seen in the positive batches. Crops grown from some of the tested batches of transplants will continue to be monitored through the growing season.

Unlike the first year of the project, where plants were collected by the grower after delivery, we collected transplant samples directly from the plant-raising nurseries. This generally resulted in lower background populations of bacteria giving increased confidence in the results.

Observations of disease progress in the field generally fell into three patterns of disease:

1. Initial and continuing low or negligible levels of black rot through the season.
2. Initial low levels of black rot which gradually increased through the season.
3. 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 (we had been unable to obtain additional seed of the particular lots concerned).

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

Pattern 2 was associated with local spread in the field from neighbouring crops with high initial levels of disease into crops with low or negligible levels in the transplants. However, race typing of isolates from the field is giving further insights. 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 airborne spread from the neighbouring heavily infected Savoy cabbage crop. However, initial race typing of isolates has indicated that the race found in the Russian kale is different from the one found in all the other crops in the field, suggesting that infection may well have resulted from a low initial level of infection in the transplants rather than spread in the field. Conversely for the Brocoletto, with a positive seed test and relatively high levels of disease in two different

fields, isolates from the field have so far proved to be a different race from the isolate from the seed. Further race typing work is underway to investigate these relationships.

The 5,000 high-health transplants produced in year 1 were followed over the winter. 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 the different timings the high-health crop had a significant weed issue due to a missed herbicide spray.

As a result of this success the approach is being repeated this year with a larger number of transplants. Transplants were again successfully produced using a 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). Again the quality 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, and will be followed through the winter.

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) and clavulanic acid (15 mg/L) and natamycin (50 mg/L).

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 company.

Variety trial

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 suspensions of the spear rot pathogen. Whereas in the first year only a single strain was used, in this second year half the plants were sprayed with the same strain as last year, and half the plants were sprayed with a recent isolate obtained from a crop in Scotland in 2020.

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) for application, with a Matabi 5 L sprayer using an Orange Evenspray nozzle at the lowest pressure consistent with even application. Five hundred mL was sprayed over a block of 10 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, one plant of each variety inoculated with each isolate (two from each tray) 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.

Transmission and spread on transplants

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 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

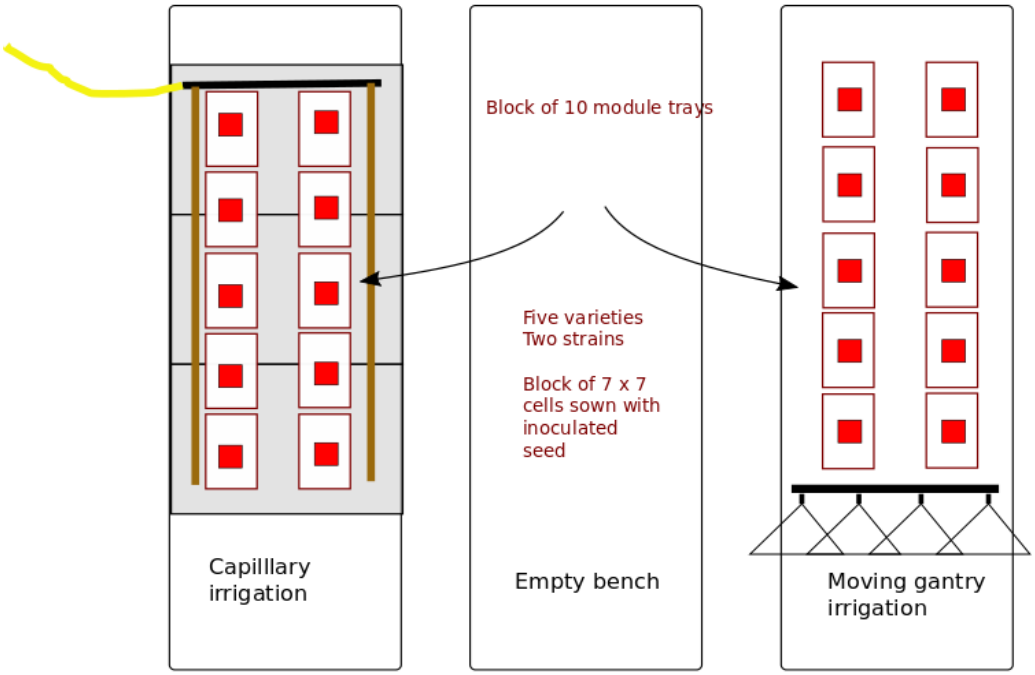

Figure 2. Experimental layout fo rthe broccolu sprear rot transmission and spread experiment

Sampling and detection on transplants

At two, and five weeks after sowing samples were collected 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.

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 last year. 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.

Results

Variety trial 2020

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

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

Transmission and spread on transplants

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 6. 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 6. Detection of spear rot pathogen strains in brassica transplants in module trays at different distances from cells sown with inoculated seed.

Watering system	Isolate	Distance (cells)	No. plants per sample	No. of samples	No. positive
Cap	2949b-2	0	4	5	4
Cap	2949b-2	3	10	5	3
Cap	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
Cap	9954	0	4	5	4
Cap	9954	3	10	5	4
Cap	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 resistance trials is extremely disappointing. This is despite inoculation of the transplants, an approach which was successful in previous trials at Wellesbourne. in the first year it was thought possible that the pathogen strain was the issue, therefore a second strain obtained from an infected crop in Scotland in 2020 was

used in 2021, and the trial was also split across two different planting dates to expose the crops to different weather conditions.

The transmission and spread experiment clearly demonstrated that transmission of the pathogen from seed to seedling is possible confirming the previous results of ~20 years ago. The experiment also demonstrated that spread of the pathogen can occur during plant raising. This is in marked contrast to the results from the first year, where no significant secondary spread was detected. Although the experiment in year 1 was designed to examine spread over longer distances (i.e. between multiple trays), there was no spread detected even at similar distances to that seen in this year's experiment.

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 and parsley seed were requested from growers.

Results

We did not receive any seed samples from growers.

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 media selective for *Pseudomonas syringae*. Cultures were mixed, with no consistent type present, and no *Pseudomonas* syringe like strains present.

Discussion

The parsley samples 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.

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

Micropropagation

A proportion of the explants were 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

Micropropagation

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.

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. 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

Discussion

We have successfully produced weaned micro-propagated cherry laurel plants, that are 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*). Work in the second year continued work begun in year 1 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 to enable us to devise plant health standards.

Materials and methods

Detection in plug plants

Samples of leaves were collected from plug 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 forty leaves

were collected from each batch and transported to the PHS lab for testing. Sub-samples were stomached in sterile saline plus tween 20, diluted and plated on selective medium BCBC (Holcroft & Roberts, 2002). Suspect colonies were sub-cultured to sectored plates of YDC, and identity confirmed by PCR. In the second year, 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 normal site.

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

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 from parental plants with no previous history of disease. Leaf samples were also tested for the presence of Xhp 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. A single plant inoculated with Xhp 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.

Results

Detection in plug plants

A total of 18 batches (70 sub-samples) of plug plants were tested/examined. The results are summarised in Table 7. In two cases (S2769 and S2803) visible symptoms were observed in

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 only one case was the pathogen detected by leaf wash plating (S2886). The values provided for % infestation (inf) 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.

For the BioPCR cases we obtained positive results for a number of sub-samples when the plating tests were negative. 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 a faint band but definitely present, 0.1 very faint band possibly non-specific. 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).

Table 7. Summary of tests on batches of Geranium plug plants for *Xanthomonas hortorum* pv. *pelargonii* (*Xhp*) from different suppliers. The shaded cells highlight the bioPCR positive samples.

Sample/ batch	Date	Supplier	Cv	N sub-samples	Total	Plating		BioPCR	
						% inf	CFU/plant	Score	% inf
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	<1.6
S2613	09/04/21	1	A	6	150	<2.0	0	0.5 (2/5)	1.7
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	0	<2.7
S2630	12/05/21	1	K	4	110	<2.7	0	2 (2/4)	2.5
S2636	27/05/21	1	B	4	110	<2.7	0	0	<2.7
S2637	27/05/21	1	A	4	110	<2.7	0	1 (1/4)	1.0
S2691	07/07/21	1	B	4	110	<2.7	0	nt	<2.7
S2713	15/07/21	4	M	3	70	<4.3	0	nt	<4.3
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	>0.1
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

* Visible symptoms.

In the spiked samples a positive plating result was obtained for 12 out of 13 samples. Positive bioPCR results were obtained for 12 out of 12 samples tested, including the spiked samples that was negative in plating.

Disease in production

Around 30 production batches were observed on up to four occasions during the season. There was marked difference in disease levels between the two sites. At Site 1, with no recent history of Geranium production, Xanthomonas leaf spots were never observed and plants remained apparently free disease 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 from current season's plugs initially appeared healthy in late May, soon after potting, but showed 100% incidence by late July. Many of these were all 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 plugs that initially appeared healthy and were sold quickly so that no further observations could be made. These were often set out on beds well away from over-wintered material.
- Later batches from current season's plugs that appear initially healthy and will be overwintered.

Spread experiment

The experiment is still on-going. The inoculated plants were placed on 19 July. Other than the inoculated plant, no disease has been observed on plants grown under protection with sub-irrigation. As at 22 Oct symptoms have been observed on 18 plants grown in the open.

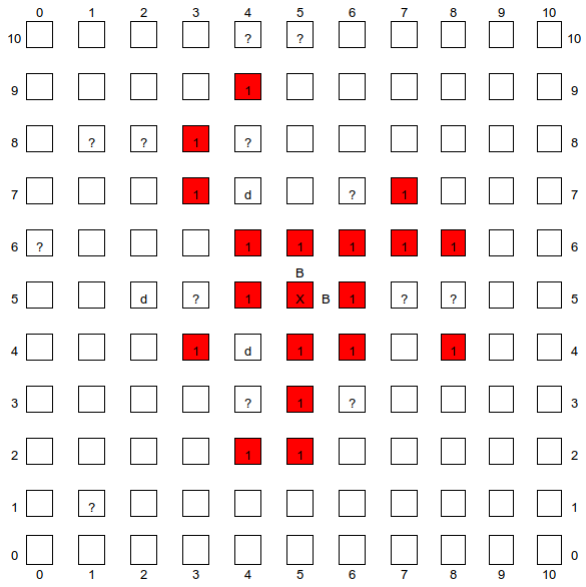

Figure 2: Map of Geranium plants with definite symptoms of Xanthomonas leaf spot on 22/10/2021. Red boxes indicate plants with symptoms. The inoculated plant is in the centre and marked with an 'X'. Pots with a ? are suspected of being infected, 'd' indicates plants that have died..

Discussion

We have demonstrated the presence of *Xhp* in some batches of plug plants on delivery to the nursery. Two of these were detected due the appearance 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. None of these batches were potted on.

Tests on most of the plug plants were negative in a conventional dilution plating assay. In the previous year all tests on plugs plants were negative, and there was concern that some of these could be false negatives. We suspected that our ability to detect low numbers of the pathogen was hampered by the presence of high background numbers of other bacteria on the dilution plates. We therefore included additional tests in the second year: we tested spiked samples of leaf wash extracts and also tested leaf wash extracts by bioPCR.

In the majority of spiked samples with relatively high numbers of Xhp added we were able to detect the pathogen by conventional dilution plating. All of these spiked samples gave a very strong band in the gels used to visualise the PCR products. In one spiked sample with relatively lower numbers of Xhp added we failed to detect the pathogen by conventional plating, but nevertheless obtained a clear positive signal in the bioPCR.

Several samples that were negative in dilution plating were also positive by bioPCR. In some cases the bioPCR results were strong positives, in others they were weak/equivocal. We cannot be certain that all of these bioPCR positives are true positives, but the results with the spiked samples suggest that where we obtained strong positive signals it is likely that these are true positives, but that the pathogen numbers may be relatively low. 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 with the stored (frozen) bioPCR extracts is planned: to repeat earlier equivocal tests under more stringent PCR conditions.

The major difference in disease levels (complete absence at one site) in production from the split batches of plugs 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 current year's production (4% incidence) was in a batch of plants derived from plugs with a positive bioPCR result.

Although the spread experiment has yet to be completed and analysed, the rate of disease spread observed from a single point source seems much lower than the apparent rapid increase observed on the production nursery.

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: (1) detection of the pathogen on bought-in plant material and (2) determining the rate of spread in order to set health standards.

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 transported to the PHS lab for testing. Sub-samples were stomached in sterile saline plus tween 20, diluted and plated on 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 (of three different varieties) 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. A single plant inoculated with *Xhp* 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.

Results

Detection in bought-in plants

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 leaves with typical symptoms 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 8. Where all sub-samples were either negative or positive the value for % Inf is the upper or lower 95% confidence limit of the estimate.

Table 8. 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	N 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

The experiment is still on-going. The inoculated plants were placed on 12 July. Other than the inoculated plant, no disease has been observed on plants grown under protection with sub-irrigation. As at 22 Oct symptoms have been observed on 25 plants grown in the open (Fig 4.)

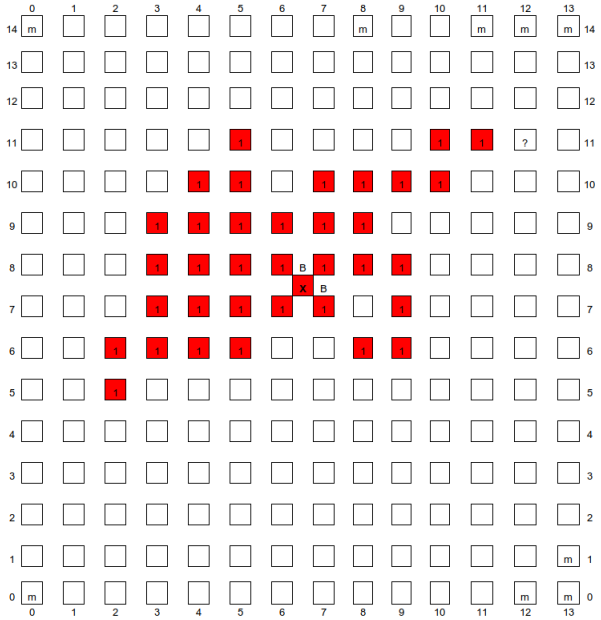

Figure 3. Map of ivy plants with definite symptoms of Xanthomonas leaf spot on 22/10/2021. Red boxes indicate plants with symptoms. The inoculated plant is in the centre and marked with an 'X'. Pots with a ? are suspected of being infected, 'm' indicates missing plants..

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) 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 might 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 an indication that it is possible to produce liners with low levels of infestation.

High Health Transplants

Previous work demonstrated that even when the 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. In the first year we successfully produced ~5000 transplants with a sub-irrigation system. These transplants were successfully planted and grown on to produce a crop which was monitored into the second year (see Black rot section). In the second year we repeated at a larger scale.

Material and methods

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

Forty '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.

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.

Plants were delivered to a commercial grower and planted alongside normal production.

Results

As in year 1, plants grew normally and produced transplants which were indistinguishable from conventionally produced transplants. Both the plant raiser and grower both reported that they were satisfied with the quality of the plants produced and delivered. A low level of downy mildew was observed at 19 days after sowing and a single spray applied. No issues were reported when planting.

At six weeks after sowing the plants were delivered to the grower and planted and will continue to be monitored until harvest.

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. 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 and 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 roots systems seem to be comparable with those of conventional transplants. The system seems to require less water, less feeding and fewer pesticide applications than conventionally produced transplants. A substantial reduction in disease and yield increase was recorded in 2021. We will continue to monitor disease in the latest batch into 2022.

Conclusions

Much of the work is still on-going at this stage, therefore final conclusions would be premature. Nevertheless, case studies have confirmed the presence of bacterial pathogens in propagation/bought-in material (e.g. seed, transplants, liners), and this has resulted in disease in the field/production, demonstrating the importance and benefits of clean starting material. The high-health brassica transplants have provided a clear demonstration of the benefits of pathogen-free transplants even when there are other sources of infection in the field. Results with geraniums have highlighted the potential limitations of the detection

systems and the perils of having multiple over-lapping batches of plants of different ages and from different sources in close proximity.

Knowledge exchange

Around 16 visits have been made to growers during the year, together with phone conversations and email exchanges of information.

The following formal presentations have been made in the year:

- Brassica Growers Association, 17 Nov 2020

Acknowledgements

We are grateful to Prof. Eric Holub of Warwick Crop Centre, Warwick University for providing some of the cultures, seed companies for providing seed, the nurserymen and growers who have provided facilities, collected samples and planted trials, and the horticultural staff at Stockbridge Technology Centre and Warwick Crop Centre.

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