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Project leader:	Dr Kim Green, ADAS
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Location of project:	ADAS Arthur Rickwood and Boxworth, Cambridgeshire; Plant Health Solutions, Warwick; HDRA, Ryton, Coventry
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Some of the results and conclusions in this report are based on a series of experiments 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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Grower Summary

Headlines

- A seed health standard appropriate for coriander bacterial blight was established.
- Hot water is the most promising seed treatment option for coriander bacterial blight; useful reductions were also obtained with thyme oil and biological control agents.
- Seed testing methods for parsley Septoria must take account of spore viability rather than presence or absence of pycnidia.
- Amistar (azoxystrobin), Signum (boscalid + pyraclostrobin) and Karamate Dry Flo Newtec (mancozeb) were effective as foliar fungicides for control of Septoria leaf spot on parsley.

Background and objectives

Parsley and coriander are the two major field-grown herb crops in the UK, with areas estimated as 1,100 ha and 1,500 ha respectively. Feedback from growers has confirmed that the priority diseases on these crops are parsley leaf spot (*Septoria petroselini*) and coriander leaf blight (*Pseudomonas syringae* pv. *coriandricola, Psc*).

Parsley leaf spot is seed-borne but can also survive on over-wintered crops and crop debris between seasons. Lesions develop on leaflets and when infection is severe can result in complete death of the foliage. However, even slight leaf spotting can render a crop unacceptable to retailers. Grower observations suggest that flat leaf parsley is more prone to leaf spot than curly leaf parsley. The disease is favoured by conditions of long leaf wetness duration and warm temperatures. Once symptoms develop, the disease can spread rapidly between beds by rain-splash and irrigation. Growers face the challenge of maintaining disease-free crops that are usually planted sequentially from April to early October.

Coriander bacterial leaf blight is a recurring problem on field-grown coriander. The disease is primarily seed-borne, but it may also survive on crop debris, although the relative importance of these inoculum sources is unknown. Disease development is probably favoured by dense plant spacing and wet conditions (e.g. regular irrigation). Seed health is key to ensuring a clean crop.

The overall objective of the proposed work was to develop integrated strategies for the management of parsley Septoria and coriander leaf blight, taking account of both seed health and field production issues. The specific objectives were to:

1. Determine appropriate seed health standards for parsley Septoria and coriander leaf blight.
2. Identify alternative methods for treatment of parsley and coriander seed, for control of *Septoria petroselini* and *Pseudomonas syringae* pv *coriandricola*, respectively.
3. Determine the efficacy of different fungicides when applied at specific timings in relation to infection events, for control of parsley Septoria.
4. Identify existing forecasting approaches that could be modified and validated to aid spray timing for management of parsley Septoria.

5. Optimise fungicide programmes for the management of parsley Septoria in inoculated field trials.
6. Prepare a fact sheet on integrated strategies for management of parsley Septoria and coriander leaf blight.

Summary of results and conclusions

Coriander bacterial blight seed transmission

Quantifying the dose-response relationship for seed to seedling transmission of the pathogen is the first step in developing a disease model which can be used to set effective seed health tolerance standards. To examine transmission a 'one-hit' theoretical model for infection was used, this makes the assumption that each individual pathogen cell is inherently capable of infection, but the probability of this occurring may be very small. The aim of the dose-response experiments is to estimate this 'one-hit' probability.

The transmission experiment in Year 1 used both naturally-infested and artificially inoculated seed to look at dose/response relationships. Transmission occurred at a lower frequency than expected and was only detected at the highest inoculum level, providing an unreliable estimate. Therefore in order to obtain a more robust estimate, the transmission experiment was repeated in the second year using the two highest plus an additional dose. Seed (fruits) were sown in '308' module trays and watered via capillary matting to avoid secondary spread. Rather than relying on the appearance of symptoms, transmission was assessed by collecting samples of plants of different sizes and analysing these for the presence of the pathogen. Transmission was detected in the two highest doses in this second experiment and the results combined with earlier data to provide an estimate of the one-hit transmission probability of 1.6×10^{-4} and a dose (scaling) parameter of 0.282. These values can be used to predict the likelihood of disease transmission for seed lots with different levels of infestation and bacterial number per infested seed, and examine these values in relation to the probability of detection for different seed health testing schemes.

Coriander bacterial blight spread trials

Quantifying the rate of disease spread in the field provides the information required for the second step in developing a disease model to set effective seed health tolerance standards. Four trials were done on the organic land at Ryton (Garden Organic/HDRA), two in Year 2 and two in Year 3. Each plot consisted of 3 x 1.8 m (5-row beds) x 10 m. To provide a point source of inoculum and simulate a single transmission event, the cotyledons of a few seedlings in the centre of the plots were inoculated with *Psc* shortly after emergence. The presence/absence of visible symptoms in each 0.5 m length of each row was then recorded at regular intervals, and used to generate a disease map.

A mathematical model was successfully fitted to the disease maps in three of the four trials These model parameters were used in defining appropriate seed health standards. In the worst case, at the time of the final recording when the crop was in flower, up to 30% of the crop was affected following a primary infection level equivalent to transmission by 1 in 15,000 seeds (fruits).

Coriander bacterial blight seed treatment

Coriander seed infested with *Psc* was treated with a range of hot water treatments, chlorine dioxide, thyme oil (white), and two biological control agents (BCAs) based on strains of *Bacillus subtilis* (Subtilex and Serenade Max).

The efficacy of the physical/chemical treatments was evaluated by testing multiple sub-samples of the treated seeds, and then using the results to provide an estimate of the infestation level. The initial results of seed tests on physically/chemically treated coriander seeds suggested that hot water was the most promising treatment and was worthy of more detailed investigation of treatment parameters, and with more seed lots. All hot water treatments gave very significant reductions in *Psc*. Initial testing suggested that the best treatment for routine use, giving the most reliable reductions in *Psc was*, 53°C for 30 min,

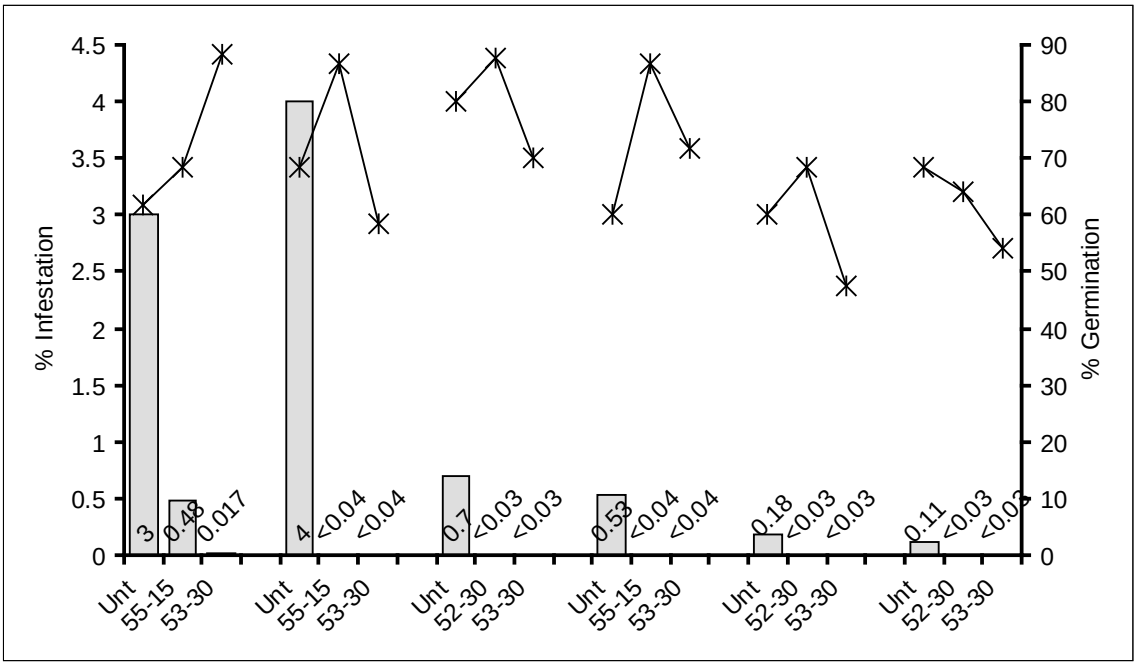

Figure 1. Effect of hot water treatment on germination and infestation levels in six coriander seed lots, naturally infested with *Pseudomonas syringae* pv. *coriandricola*. Bars represent the infestation level, lines represent germination. Unt = untreated; 55-15 = 55°C for 15 min; 53-30 = 53°C for 30 min; etc.

without any reduction in germination. However, when tested on a wider range of seed lots, some seed lots suffered a reduction in germination; reducing the temperature to 52°C in these seed lots preserved germination whilst still reducing *Psc* to undetectable levels. Except for one seed lot, the reductions achieved by 53 or 52°C for 30 min would be sufficient for the seed lots to achieve the proposed seed health standard.

In initial tests, Thyme oil at 10% also gave significant reductions in *Psc*, albeit with an adverse effect on germination. Subsequently it was tested at lower concentrations: these had less impact on germination, but also had less impact on *Psc.* Given the apparent success of thyme oil for other host/pathogen combinations it could be worth pursuing alternative formulations and treatment durations.

Chlorine dioxide at the concentrations used (100 and 500 ppm) appeared to have no effect.

Because of the presumed ways in which the BCAs work, seed testing cannot be used to test their efficacy. The two BCAs (Subtilex and Serenade Max) were therefore evaluated in glasshouse transmission experiments using both inoculated and naturally infected seed lots. This requires a lot more effort than seed testing and limits the number of experimental units and total numbers of seeds (effectively 2,000 v 6,000) which can be examined and hence the 'statistical power' of the data analysis. Nevertheless clear indications of reductions in transmission and bacterial populations were obtained for both BCAs. This is also consistent with the results obtained in FV 335 (Roberts 2009) for another bacterial disease (black rot of brassicas).

Coriander seed health standards

Transmission, spread, and seed testing models were combined to examine the risks of significant disease development for a range of seed infection levels both in terms of the % of seeds infested and the numbers of bacteria on those infested seeds. Results for some example scenarios are shown in **Table 1** and suggest that a seed health tolerance standard of 0.03% and test sensitivity of 900 CFU is appropriate for coriander seed used for fresh leaf production in the field. This translates to testing 3 sub-samples of 3,000 seeds by the 'PHS method'. A different, more stringent, standard may be appropriate for seed used for seed/spice crops or protected pot-grown production.

Table 1. Probability of transmission, and coriander bacterial blight disease incidence for an area of approx. 0.36 ha sown with 1 million seeds with infestation levels from 0.003% to 0.1% and bacterial numbers from 10^2 to 10^5 CFU per infested seed, together with the probability of detection in seed tests on either 1 x 3,000 seeds or 3 x 3,000 seeds.

Seed inf.			Prob.	Incidence	Pr +ve test[2]		Overall risk[3]	
1 in	% inf	CFU	Trans.[1]	Max %	1 x 3k	3 x 3k	1 x 3k	3 x 3k
30,000	0.003	1 x 10^2	0.019	19	0.03	0.08	0.02	0.02
30,000	0.003	1 x 10^3	0.037	19	0.09	0.25	0.03	0.03
30,000	0.003	1 x 10^4	0.069	19	0.10	0.26	0.06	0.05
30,000	0.003	1 x 10^5	**0.128**	19	0.10	0.26	**0.12**	0.09
15,000	0.007	1 x 10^2	0.038	33	0.06	0.16	0.04	0.03
15,000	0.007	1 x 10^3	0.072	33	0.18	0.44	0.06	0.04
15,000	0.007	1 x 10^4	**0.133**	33	0.18	0.45	**0.11**	0.07
15,000	0.007	1 x 10^5	**0.240**	33	0.18	0.45	**0.20**	**0.13**
10,000	0.010	1 x 10^2	0.057	45	0.08	0.23	0.05	0.04
10,000	0.010	1 x 10^3	**0.106**	45	0.25	0.58	0.08	0.04
10,000	0.010	1 x 10^4	**0.193**	45	0.26	0.59	**0.14**	0.08
10,000	0.010	1 x 10^5	**0.337**	45	0.26	0.59	**0.25**	**0.14**
5,000	0.020	1 x 10^2	**0.111**	70	0.16	0.40	0.09	0.07
5,000	0.020	1 x 10^3	**0.201**	70	0.44	0.82	**0.11**	0.04
5,000	0.020	1 x 10^4	**0.349**	70	0.45	0.83	**0.19**	0.06
5,000	0.020	1 x 10^5	**0.561**	70	0.45	0.83	**0.31**	0.09
1,000	0.100	1 x 10^2	**0.444**	95	0.57	0.92	**0.19**	0.03
1,000	0.100	1 x 10^3	**0.674**	95	0.94	1.00	0.04	0.00
1,000	0.100	1 x 10^4	**0.883**	95	0.95	1.00	0.04	0.00
1,000	0.100	1 x 10^5	**0.984**	95	0.95	1.00	0.05	0.00

[1] Probability of at least one infected seedling in the block. Values >0.1 in bold.
[2] Probability of detection in seed test performed according to PHS standard method.
[3] The overall risk is the probability of a negative test result combined with the probability of transmission. Shaded values are considered to represent an unacceptable risk.

Parsley seed health

Parsley seed was examined to determine the nature of infection by *S. petroselini,* using seven seed lots reported to be infected with *S. petroselini*. Pycnidia of *S. petroselini* were visible on seed from all seven batches tested, with one batch containing 40% of seeds with pycnidia. Spore release from pycnidia was observed for five seed lots, however subsequent germination of released spores (indicating pathogen viability) was observed for only two seed lots. Examination of washings from the seed lots showed that spores of *S. petroselini* can be present on seeds both with and without visible pycnidia. The results demonstrate that neither the percentage of seeds with pycnidia nor the percentage of seeds showing spore release from pycnidia give a useful measure of pathogen viability or subsequent risk to a parsley crop. Moreover, a seed lot with pycnidia could pose little risk, while a seed lot that is apparently healthy (i.e. without visible pycnidia) could contain viable spores. These findings show that development of a standard seed testing method for parsley Septoria must take account of the viability of spores on seed lots, rather than relying on presence of pycnidia and spores alone.

The frequency of pathogen transmission was studied using seven parsley seed lots. Seedlings in module trays were exposed to conditions conducive for Septoria in controlled environment cabinets and then monitored for lesion development. Transmission of *S. petroselini* to seedlings was demonstrated using only one seed lot for which the pathogen was previously shown to be viable. Estimates of the one-hit probability of transmission (probability of transmission of one spore on one seed) varied, with values of 9.0×10^{-5}, 8.7×10^{-5} or 6.2×10^{-5}, depending on whether primary infection foci were considered at the seedling, cell or cluster level, respectively. To further verify this rate and to determine dose response, transmission studies were done with parsley seed artificially infested with varying doses of viable *S. petroselini* spores, however, no symptoms of Septoria developed on seedlings from these seed lots.

A disease spread experiment was sited in a polytunnel at ADAS Arthur Rickwood, Cambs. The crop was established using modules planted almost continuously along the row (3 cm spacing) with 20 cm between rows, giving an area of 21 rows and 7 m length. The crop was overwintered then cut back to stem bases. One day after crop cutting (4 June 2009), a potted plant of parsley var. Bravour with typical symptoms of Septoria was placed as an infector plant in the centre of the experimental area. To provide conditions that were conducive for disease development, overhead irrigation was applied for 10 min daily for the next 8 weeks. The trial area was monitored regularly for development of parsley Septoria symptoms, by determining the number of plants infected in quadrats of 3 rows wide (0.6 m) by 0.5 m length. Symptoms of Septoria were first observed 12 d after placing the infector plant in the experimental area. For the first 2 weeks after introduction of the infector plant, disease symptoms all occurred less than 0.5 m from the infection point. By 5 weeks, symptoms had spread up to 3 m along the length of the trial area. This had increased to spread up to 3.5 m from a point source by 7 weeks (Figure 3).

The disease spread experiment was useful in demonstrating the extent to which parsley Septoria can spread under environmental conditions that are high risk for disease development. However, because of limited results from the transmission experiments,

models could not usefully be fitted to transmission and disease spread data to further define seed health standards for parsley Septoria.

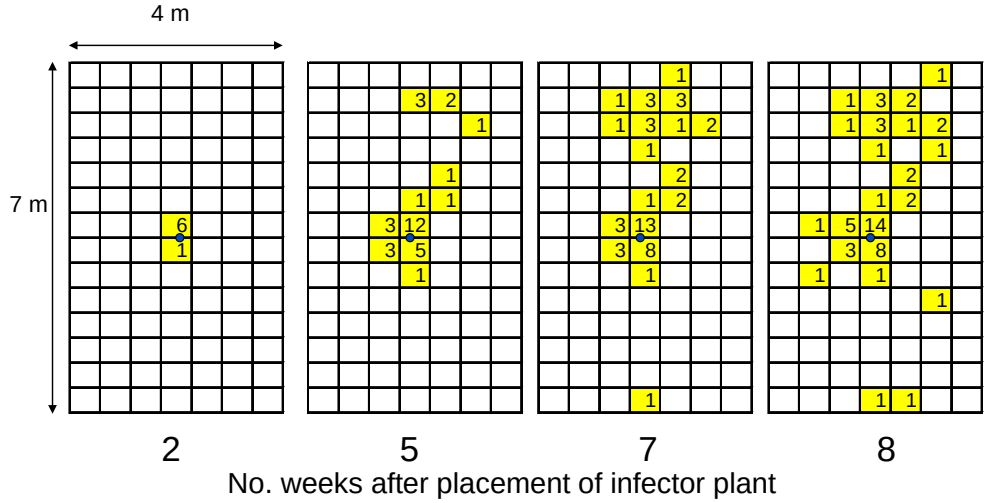

Figure 2. Spread of septoria from a single infection focus on parsley, Cambs 2009

Parsley seed treatment

Parsley seed can currently be treated for Septoria using a warm water thiram soak. Fungicides approved for foliar applications on parsley are not permitted for use on seed. Alternative seed treatments for parsley Septoria could not be evaluated in this project due to lack of seed lots with viable *S. petroselini.* A previous EU-funded project (STOVE) looked specifically at alternative seed treatments for control of *Septoria petroselini*. Schmitt *et al*. (2008) reported that many of the methods applied had a beneficial effect on seed germination and reduced disease by *Septoria*, including use of hot water, aerated steam, BA 2552 (*Pseudomonas chlororaphis*), *Bacillus subtilis* K 3 and thyme oil.

Foliar fungicides for parsley Septoria

Foliar fungicides were evaluated for control of parsley leaf spot, using curly leaf parsley artificially inoculated with S. petroselini in trays in a glasshouse. The following fungicide products significantly reduced the incidence and severity of parsley Septoria caused by S. petroselini: Amistar (azoxystrobin), Signum (boscalid + pyraclostrobin), Folicur (tebuconazole) and Karamate Dry Flo Newtec (mancozeb). Mancozeb was the most effective fungicide tested, reducing mean disease incidence to 14% at 34 days after inoculation, compared to 100% in the untreated control. Amistar was most effective when applied 2 days before inoculation, while Karamate Dry Flo Newtec gave excellent control even when applied 5 days before inoculation.

When the same fungicides were applied to Septoria lesions containing mature pycnidia, all fungicides tested except Switch (cyprodinil + fludioxonil) reduced % spore germination, with Amistar and Signum being particularly effective. This result indicated that application of a strobilurin product to mature Septoria lesions could at least delay secondary spread of the disease by limiting germination of spores present in pycnidia.

Fungicide programmes for control of parsley Septoria were evaluated using an artificially inoculated polytunnel crop. Three spray programmes were evaluated (Karamate Dry Flo Newtec x 3, or Amistar x 2, then Karamate Dry Flo, or Signum x 2 then Karamate Dry Flo; all with SOLAs for outdoor parsley), either commencing before or after an infection event. All programmes were effective in reducing Septoria incidence (Figure 4) and severity, when programmes commenced before infection had occurred. Use of Karamate Dry Flo Newtec close to harvest should be avoided because of the risk of visible spray deposits on leaves.

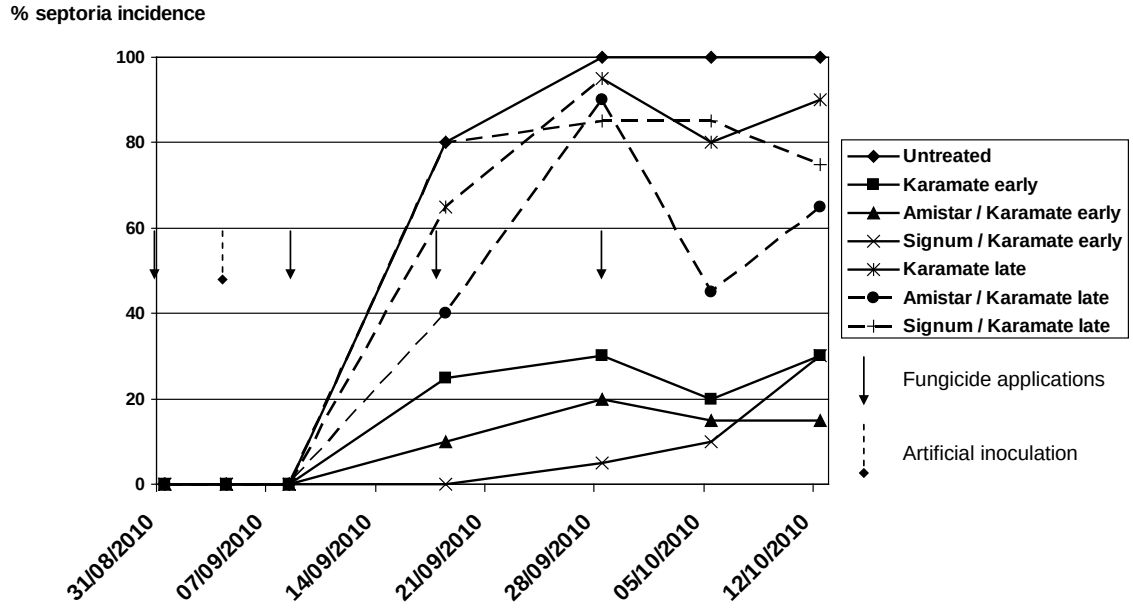

Figure 3. Development of parsley septoria following fungicide applications and artificial inoculation with *Septoria petroselini*, Cambs 2009.

Potential forecasting approaches for parsley Septoria

Knowledge of environmental conditions that are favourable or unfavourable for the development of parsley Septoria can help to minimise spray applications. A literature review was done to summarise known information on environmental conditions conducive for the development of parsley Septoria, and possible forecasting approaches.

Key points from literature on the impact of environmental conditions on the development of parsley Septoria are as follows:

- The mean number of lesions per unit leaf area increased with inoculum concentrations from 10^4 to 2×10^6 conidia ml^{-1}.
- The optimum temperatures for lesion development were 20 and 23°C. At those temperatures, the optimum leaf wetness duration was 72 h.
- Low levels of Septoria blight on parsley can develop across a wide temperature range.
- Under optimum conditions, symptoms of Septoria can develop 9 days after inoculation.

There are no models that have been developed specifically for predicting the development of Septoria on parsley. Of other models reviewed (particularly those previously developed for celery Septoria), the Tom-Cast system has the advantage that it has been validated for use in a range of crop/disease situations, and has been shown to enable reduced spray numbers

in certain seasons while still maintaining marketable quality. It requires relatively inexpensive equipment, provides a straightforward output and has been implemented by growers. The disadvantage of the Tom-cast model is that it relies on previous weather conditions rather than forecast conditions, such that fungicides with strong curative activity (not available for parsley) would be required. On a short duration crop such as parsley, for which quality standards are high, growers would be advised to apply protectant sprays of fungicides effective for parsley Septoria control when high risk conditions are forecast (particularly prolonged wet periods). Spray intervals can be extended or numbers of applications reduced during dry conditions because Septoria risk is lower.

Financial benefits

The project has addressed two key diseases of parsley and coriander. Development of seed health standards and alternative seed treatment methods for coriander will help to improve seed quality, thus reducing crop losses due to bacterial blight. Further information on the seed-borne nature of parsley Septoria will enable more appropriate testing for this pathogen and reduced crop losses. An evaluation of fungicide efficacy and appropriate product timing will allow effective parsley Septoria control during field production, while minimising spray applications.

Action points for growers

- It is not possible to guarantee that coriander seed is completely free from *Pseudomonas syringae* pv. *coriandricola* (*Psc*).
- Where possible growers should request coriander seed which has been tested for *Psc* to the recommended tolerance standard of <0.03% or as agreed with the supplier.
- Coriander seed which does not meet the standard may be hot-water treated and re-tested to ensure compliance with the standard.
- For parsley seed, request that seed testing methods for parsley Septoria include a check for pathogen viability, rather than just presence or absence of pycnidia.
- Parsley seed testing positive for *Septoria petroselini* can be treated using a warm water thiram soak.
- Broad spectrum disinfectants/biocides are not permitted for use as seed treatments for coriander or parsley.
- Amistar, Signum and Karamate Dry Flo Newtec (all with SOLAs for outdoor parsley) can be used as foliar fungicides for the control of parsley Septoria. Use of Karamate Dry Flo Newtec close to harvest should be avoided because of the risk of visible spray deposits on leaves.

Science Section

Introduction

Parsley and coriander are the two major field-grown herb crops in the UK. Areas of these crops were recently estimated as 1,100 ha for parsley and 1,500 ha for coriander. Feedback from outdoor herb growers has confirmed that the priority diseases on these crops are parsley leaf spot (*Septoria petroselini*) and coriander leaf blight (*Pseudomonas syringae* pv. *coriandricola*, *Psc*).

Parsley leaf spot is seed-borne but can also survive on over-wintered crops and crop debris between seasons. Lesions develop on leaflets and when infection is severe can result in complete death of the foliage. However, even slight leaf spotting can render a crop unacceptable to retailers. Grower observations suggest that flat leaf parsley is more prone to leaf spot than curly leaf parsley. The disease is favoured by conditions of long leaf wetness duration and warm temperatures. Once symptoms develop, the disease can spread rapidly between beds by rain-splash and irrigation. Growers face the challenge of maintaining disease-free crops that are usually planted sequentially from April to early October.

Coriander bacterial leaf blight is a recurring problem on field-grown coriander and has also been reported in protected pot-grown production. The disease was first seen in the UK in 1967, but was not formally reported in the scientific literature until 1980 (Taylor and Dudley 1980). It has also been reported in Australia, Germany, Hungary, Mexico, Spain and the USA. The disease is also described as umbel blight and seed decay in some of these reports. The disease is primarily seed-borne, it may also survive on crop debris, although the relative importance of these inoculum sources is unknown. Disease development is favoured by dense plant spacing and wet conditions (e.g. regular irrigation). Seed health is key to ensuring a clean crop.

As both diseases are seed-borne, the use of clean seed is vital for their control, however seed health tolerance standards have not been defined and effective seed treatment methods are not available. Knowledge of the relationships between seed infestation levels and disease in the crop are essential for effective disease management via a clean seed policy. Seed treatments to reduce inoculum levels may also be effective when clean seed is not available.

A range of fungicidal active ingredients currently have approval for use on outdoor herbs, mainly as specific off-label approvals (SOLAs). Products such as Amistar (azoxystrobin), Signum (boscalid + pyraclostrobin), Folicur (tebuconazole) may be effective against parsley Septoria. Despite the availability of appropriate fungicides for parsley Septoria, growers still report disease outbreaks, suggesting that the timing of specific fungicide applications is not being optimised in relation to infection events. There is also a need to implement strategies for fungicide use that minimise the risk of developing pathogen resistance when products from the same fungicide group are used repeatedly. In order to meet consumer demands, growers need to minimise fungicide use while still producing high quality crops. Knowledge of (i) appropriate timing of fungicides with different modes of action, in relation to infection

events, and (ii) environmental conditions that are favourable or unfavourable for disease development, can help to minimise spray applications.

The overall objective of the proposed work is to develop integrated strategies for the management of parsley Septoria and coriander leaf blight, taking account of both seed health and field production issues. The specific objectives are:

1. Determine appropriate seed health standards for parsley Septoria and coriander leaf blight.
2. Identify alternative methods for treatment of parsley and coriander seed, for control of *Septoria petroselini* and *Pseudomonas syringae* pv *coriandricola*, respectively.
3. Determine the efficacy of different fungicides when applied at specific timings in relation to infection events, for control of parsley Septoria.
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5. Optimise fungicide programmes for the management of parsley Septoria in inoculated field trials
6. Prepare a fact sheet on integrated strategies for management of parsley Septoria and coriander leaf blight

The results of work done in the first and second years of the project have been reported previously (Green and Roberts 2008; Green and Roberts 2009).

This report consolidates the results of work done during the final year of the project together with the information contained in the previous reports.

Developing appropriate seed health standards for coriander

Introduction

As a seed-borne bacterial disease the most effective means of control is to use 'clean' seed'. However, as seed health tests are necessarily done on a sample of seed, there is a need to define the tolerance standard and analytical sensitivities which need to be achieved by any testing program. Thus the aim of this part of the work was to define seed health standards for coriander seed which would minimise the risk of damaging levels of disease in the field. It is important to note the term 'minimise the risk' as no seed health assay can be considered 100% reliable due to sampling and other sources of variability.

The required seed health standard depends on:

- the rate of transmission from seed to seedling;
- the rate of disease spread in the field;
- economic damage in relation to disease levels

Further information can be found in (Roberts 2006)

The approach used in this work was to estimate the rate of transmission from seed to seedling in glasshouse dose-response experiments and to estimate the rate of spread in a series of field trials.

Materials and Methods

Source of seeds

Contacts were made with a number of seed companies supplying coriander seed and requests made for samples of seed lots in both years 1 and 2. The seed samples were tested for *Psc* and the level of infestation quantified. Larger quantities of seed identified as being useful to the project (either because of apparent freedom from disease or with high levels of infestation) were then requested, and infestation levels re-checked.

Coriander seed testing

Seeds were tested according to the methods developed by Plant Health Solutions for commercial routine testing of coriander seed for *Psc*. A brief description of the method follows. Sub-samples of up to 5,000 seeds are soaked overnight at 4-5°C then stomached for 5 min. The resulting extract is then diluted and plated on two selective media (P3 and S4). Plates are incubated for 3-4 d at 25°C and the numbers of suspect *Psc* colonies recorded. Suspect *Psc* colonies are then sub-cultured to non-selective media and their identity confirmed by inoculation into coriander seedlings.

In some cases, individual seeds were placed on the surface of plates of the selective agar media, and incubated for 3 d at 25°C. The presence of suspect colonies of *Psc* around the individual seed was taken to indicate infestation. Suspect colonies were sub-cultured and identity confirmed as above.

In order to quantify infestation levels it was necessary to test repeated sub-samples of seed of varying sizes for each seed lot. The numbers of positive/negative sub-samples for a given

sub-sample size were then used to obtain a maximum likelihood estimate of the proportion of seeds infested using the STPro™ computer program (Ridout and Roberts 1995).

Coriander seed transmission

Experiments were done during the first year of the project and then partially repeated in the second year to increase the reliability of the data.

Seed inoculation

Psc isolate 9021 was grown for 48 h at 25°C on plates of PAF (Difco Pseudomonas Agar F). A large loopful of growth was suspended in 20 ml of SDW (sterile distilled water), and a series of five fivefold dilutions prepared, plus two further tenfold dilutions to enable inoculum counts. The numbers of bacteria in the inocula were estimated using the drop method of Miles and Misra (1933) using 4 x 20 µl drops on plates of PAF.

Aliquots (8 ml) of each of the fivefold dilutions were added to 20 ml of SDW in 250 ml conical flasks. Aliquots (15 g) of coriander seed (fruits, seed lot S1046, previously tested and found to be free from infestation) were added to the flasks. The volume of inoculum was sufficient to just cover the seeds. Flasks were then shaken to mix and wet the seeds. After approximately 5 min half were subjected to vacuum for 5 min then released. Then after approximately 15 min total, seeds were poured out onto absorbent paper towel in a tray. The paper towel was replaced after approximately 10 min. and then seeds allowed to dry at room temperature for 2 days.

After allowing to dry, the seed was packaged in seal easy bags and stored in the fridge.

The dose of bacteria on the seeds was estimated both before and after sowing by testing small sub-samples or individual seeds as described previously.

The dose of bacteria on the seeds was estimated the day after sowing, and one week later by testing small sub-samples or individual seeds as in Year 1.

Seed sowing

‘308’ module trays were loosely filled with Bulrush Modular Organic Compost, levelled and compressed slightly. Coriander seeds were sown (1 fruit per cell) and covered with sieved compost. Trays were then set out on capillary matting on glasshouse benches. Trays were overhead-watered immediately following sowing, all further watering was then via capillary matting to minimise the risk of plant-to-plant spread.

The glasshouse regime was set as day/night min. 18/15°C and vent at 20/17°C. Temperature was monitored continuously using a Tinytag temperature logger. In most cases two 308 trays were sown for each inoculum dose, but in the first year one tray was also sown with each of five naturally infested seed lots (S1042, S1043, S1044, S1047, S1048).

Assessment

Rather than waiting for symptom expression, transmission was estimated by determining the proportion of seedlings contaminated with the pathogen. Three weeks after sowing, samples of plants were collected from cells in each treatment. All plants in a cell were collected (i.e. 1 or 2 depending on germination of the two seeds in each fruit sown). Six samples of varying

sizes (2, 2, 2, 14, 44, and 70 cells in Year 1; 7 to 50 cells in Year 2) were collected from each treatment. These sample sizes were designed to ensure that estimates of the contamination level could be obtained within the prior range of 0.5% to 90% in Year 1 and 0.1% to 20% for the two lowest doses and 1% to 40% for the highest dose in Year 2. The optimised design was obtained using a Fortran program specially written for the purpose (Ridout 1995). Samples were collected by cutting the stems with scissors just below the cotyledons and were placed directly into new stomacher bags. Within a tray, samples were collected systematically to ensure coverage of the whole tray. To minimise the potential for cross contamination, samples were collected from trays in order of inoculum concentration (lowest to highest) and scissors and hands were disinfected between each treatment using 70% isopropanol. Following collection, samples were stored in a fridge for up to 2 d before processing.

To process each sample, saline plus 0.02% Tween 20 was added to the stomacher bags (1.5 ml to 2 plant samples, 0.5 ml per plant for larger samples), and the plants were then stomached for 5 min. The resulting extract was then diluted and 0.1 ml of each dilution and the original extract were spread on the surface of plates of P3 selective medium. Plates were then incubated for 3-4 days at 25°C and the numbers of suspect *Psc* recorded. Suspect *Psc*. colonies were then sub-cultured and their identity confirmed by inoculation into coriander seedlings.

Spread in the field

Following further seed testing, a 'high health' seed lot (S1041, <0.02% infestation) was identified and used for both drilling dates in both years.

Four trials were done in the organic field trial area at Garden Organic (HDRA, Ryton Organic Gardens). Plots consisted of 3 x 1.8 m beds x 10 m. Prior to drilling, land was rotovated and made up into standard beds. The day before drilling, plots were irrigated if necessary, to ensure soil was at field capacity. Seed (fruits) were sown in 5 rows (approx. 30 cm spacing) per bed at a rate of 100 seeds per m of row (approx. equivalent to 20 kg/ha) using an Earthworks hand drill with the 'Beets' plate fitted and with the outlet modified to give an even distribution along the row.

Plots were weeded by hand hoeing between rows. Irrigation was applied as necessary using Agridor 900-240 spray heads which delivered approx 1.4 mm/h.

Shortly after emergence (11-14 d after sowing), five seedlings in the centre of the plot were inoculated with *Psc* (isolate 9021) to provide a 'point' source of inoculum. The bacterium was grown for 48 h at 25°C on a plate of PAF agar, and seedlings inoculated by stabbing the cotyledons with an insect pin which had been dipped in the bacterial growth on the plate.

Weather data was recorded using a Spectrum Watchdog 2000 Series weather station (EnviroMonitors, East Sussex). Data was recorded at 10 min intervals.

Crops were monitored regularly for signs of disease spread, and the incidence and severity of disease recorded in each 0.5 m section of each row.

In Year 2, the first trial was drilled on 27 June 2008 and the second was drilled on 29 August 2008. In Year 3, the first trial was drilled on 08 June 2009 and the second on 14 August 2010.

Statistical analyses

The proportions of infested seeds in infested seed lots and their 95% confidence limits were estimated by maximum likelihood methods using the STPro™ seed test analysis program (Ridout and Roberts 1995). The mean numbers of bacteria on seeds was estimated by fitting a Generalised Linear Model to the plate counts using a Poisson distribution, log link function, with dilution as an offset and the number of seeds in the sample as a weighting factor.

Transmission rate was estimated by fitting a Generalised Linear Model (GLM) to the presence/absence of *Psc* in each sample using a complementary log-log link function and sample size as an offset. The model was fitted using the Genstat statistical analysis program (Payne *et al.* 2005).

Rate of spread was examined by fitting a generalised non-linear model containing both spatial and temporal parameters to the data using the FIT directive in Genstat.

Example scenarios for seed health standards

The models for seed to seedling transmission and spread in the field were used to examine the potential outcomes from sowing 1 million seeds (equivalent to an area of *ca.* 0.36 ha) of seed lots with different infestation levels, both in terms of the proportion of infested seed and the mean numbers of *Psc* on infested seeds. Parameter values used were those obtained in the transmission experiments and from the field trial with the greatest spread (Trial 3).

For each seed lot the probability of obtaining a positive test result for a sub-sample is a function *F* of the proportion infested, θ, the sub-sample size, *m*, and the test sensitivity (p_s), i.e. $p_+ = F(\theta, m, p_s)$, where:

$$F(\theta, m, p_s) = \sum_{j=1}^{m} bi(j, m, \theta) \times \left(1 - (1 - p_s)^j\right)$$

and *bi*() is the individual term in the binomial expansion. The test sensitivity p_s, is dependent on the mean number of bacteria on an infested seed, *x*, the volume of liquid in which they are suspended/extracted, *V*, and the volume of liquid plated, *v*, and is calculated as:

$$p_s = 1 - \exp(-v\frac{x}{V})$$

Thus, the probability of positive test results being obtained was calculated for different sample sizes, numbers of sub-samples, etc. Finally an overall measure of risk was obtained by multiplying the probability of transmission with the probability of a negative test result.

Results

Coriander seed testing

Eight out of the fourteen coriander seed lots examined were found to be infested with *Psc*. Infestation levels together with upper and lower confidence limits, as estimated using STPro are shown in , and ranged from 0.4 to 5% in infested seed lots. For lots where *Psc* was not

detected an upper confidence limit is provided. These estimates are based on the results of tests on over 150 sub-samples of seeds.

Table 1. Results of seed tests on coriander seeds naturally infested with *Pseudomonas syringae* pv. *coriandricola*

Sample No	% of seeds infested			Numbers of bacteria per seed (weighted mean)		
	Mean	95% confidence limits		Mean	95% confidence limits	
		Lower	Upper		Lower	Upper
S1040	<0.03	0.00	0.03	<0.6	0.0×10^0	0.6×10^0
S1041	<0.012	0.00	0.012	<0.6	0.0×10^0	0.6×10^0
S1042	0.7	0.12	4.6	3.3×10^3	1.9×10^3	6.0×10^3
S1043	3.9	0.37	22	5.9×10^3	3.7×10^3	9.3×10^3
S1044	5.0	0.40	33	2.2×10^3	1.3×10^3	3.9×10^3
S1045	4.4	0.38	26	1.6×10^3	0.9×10^3	3.1×10^3
S1046	<0.015	0.00	0.015	<0.6	0.0×10^0	0.6×10^0
S1047	0.7	0.12	4.6	5.9×10^2	2.0×10^2	17×10^2
S1060	>0.009	0.009	100	1.4×10^1	0.5×10^1	3.8×10^1
S1062	0.4	0.03	2.4	1.1×10^2	4.6×10^1	2.8×10^2
S1071	>0.02	0.02	100	2.8×10^4	2.0×10^4	4.0×10^4
S1072	4.4	1.40	11	2.2×10^4	1.5×10^4	3.2×10^4
S1073	3.9	1.10	11	9.0×10^3	6.1×10^3	1.3×10^4
S1076	<0.05	0.00	0.054	<0.6	0.0×10^0	0.6×10^0

Coriander Seed Transmission

In Year 1, the inoculum concentrations used ranged from 6.1×10^3 to 3.9×10^6 CFU/ml and resulted in received doses per seed ranging from 1.8×10^1 to 6.9×10^4 CFU per seed. In the additional experiment, the doses of bacteria per seed ranged from 2.1×10^3 to 6.5×10^6 CFU per seed. Contaminated seedlings were detected for the two highest doses. The proportion of seedlings contaminated was estimated using the STPro program and results are shown in .

Table 2. Relationship between mean dose of bacteria per seed and transmission from seed to seedling for coriander seeds inoculated or infested with *Pseudomonas syringae* pv. *coriandricola*

Treat Code	Dose (CFU/seed)	% Infection	95% confidence limits	
			Lower	Upper
Vacuum inoculated(Year 1)				
4V	6.9E+04	0.011	0.00059	0.049
1V	6.0E+04	0.033	0.0050	0.14
2V	2.6E+04	<0.022	0	0.022
3V	8.1E+03	<0.022	0	0.022
5V	2.7E+02	<0.022	0	0.022
Vacuum inoculated (Year 2)				
0V	6.5E+06	0.032	0.0051	0.11
1V	3.8E+06	0.0059	0.00034	0.026
2V	2.2E+03	<0.014	0	0.014
Dip inoculated				
1	1.4E+04	<0.022	0	0.022
2	2.0E+03	<0.022	0	0.022
3	2.9E+02	<0.046	0	0.046
4	7.1E+01	<0.022	0	0.022
5	1.8E+01	<0.022	0	0.022
Naturally infested				
1042	3.3E+03	<0.046	0	0.046
1043	5.9E+03	<0.046	0	0.046
1044	2.2E+03	<0.046	0	0.046
1045	1.6E+03	<0.046	0	0.046
1047	5.9E+02	<0.046	0	0.046

The data from the two experiments were combined to obtain an estimate of the one-hit probability of transmission. The value was estimated using Genstat™ by fitting a GLM to the data for vacuum inoculated seeds for both experiments. It was estimated to be 1.6×10^{-4}, with a dose coefficient of 0.282.

Spread in the field

Trial 1 (Year 2)

In the first crop, seedlings began to emerge approx. 7 d after sowing, with good emergence in all rows of each bed. Disease symptoms (brown lesions) were clearly visible on the inoculated seedlings by 12 d after inoculation (25 d after sowing, 2TL). The first convincing symptoms outside of the inoculated plants were seen 40 d after sowing at which point the crop was beginning to bolt. A map of showing the pattern of disease at the final assessment is shown in Figure 1; approx. 10% of quadrats had disease symptoms. Disease symptoms were only observed in the central bed.

Trial 2 (Year 2)

Drilling of the second crop was considerably delayed due to wet weather preventing the preparation of seed bed. Seedlings emerged well, with good emergence in all rows by 10 d after sowing. Severe slug damage occurred in one of the edge beds, with almost all seedlings destroyed. It also appeared that the inoculated seedlings in the middle bed were selectively eaten, therefore a repeat inoculation was necessary 21 d after sowing. In addition, as no spread had been observed between beds in the first sowing, a few central plants in the remaining bed were also inoculated. Some possible spread was observed on plants immediately neighbouring the primary infectors 53 d after sowing (21 Oct), however, due to the general deterioration in plant quality it became very difficult to be certain of symptoms and the trial was abandoned.

Trial 3 (Year 3)

Seedlings emerged by 9 d after sowing, with good emergence throughout. Inoculated seedlings with symptoms were placed in the plot 14 d after sowing. The first evidence of spread (i.e. symptoms on non-inoculated plants) was seen 37 d after sowing (23 d after introduction). A map showing the distribution of disease is show in Figure 1. The crop began to bolt 44 d after sowing.

Trial 4 (Year 3)

Seedlings began to emerge 7 d after sowing, with good emergence in all rows by 10 d. Seedlings in the centre of the plot were inoculated 12 d after sowing. Due to a lack of convincing symptoms these plants were re-inoculated 28 d after sowing. The first symptoms on non-inoculated plants were seen 55 d after sowing (early October). The crop and recording were abandoned in December following heavy frost damage. Some of the earliest disease symptoms were seen at the edge neighbouring the location of the earlier sown trial crop.

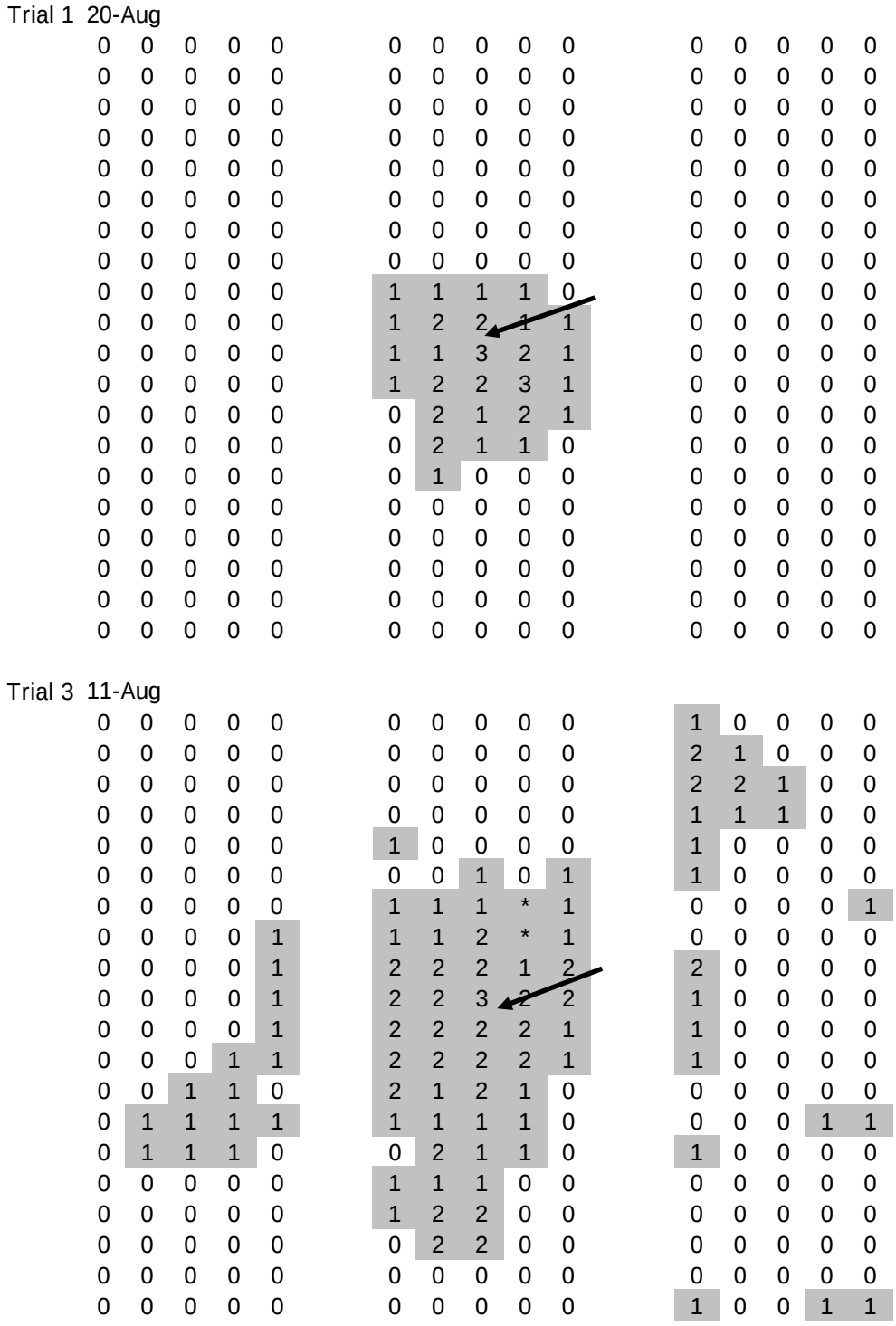


Figure 1. Maps showing the distribution of bacterial blight symptoms, at the start of bolting in trials (field plots 10 m x 3 5-row beds) to examine the spread of *Pseudomonas syringae* pv. *coriandricola.* The arrow indicates the position of the primary infector plants. Each number represents the disease severity score (0-4 scale) in a 0.5 m section of row.

Model fitting

A generalised non-linear model was fitted to the mapped symptom data. The model was in the form:

$$\text{logit}(p) = a - b\ln[c + d] + rt$$

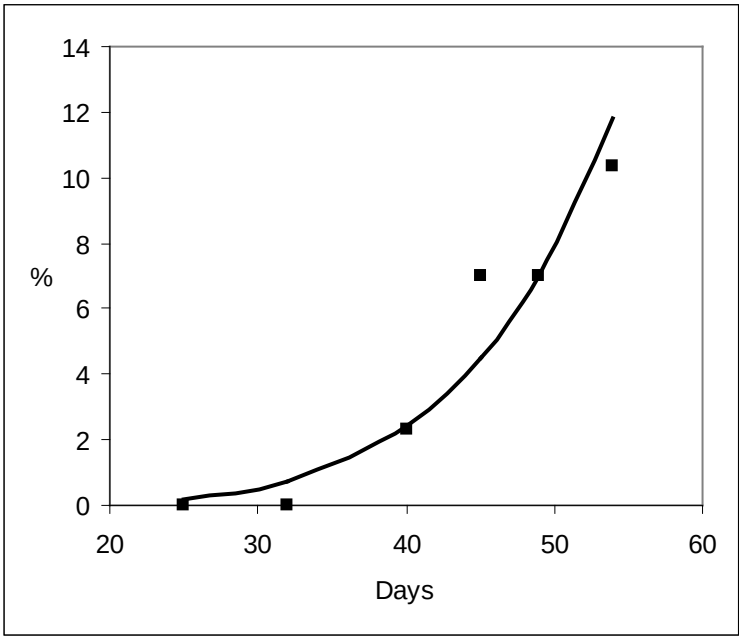
where *p* is the proportion of quadrats with symptoms, *a* is an intercept parameter, *b* is the disease gradient, *d* is the distance from the source (primary infector), *c* is a truncation factor that allows for calculation of a finite level of disease at the source, *t* is time in days since sowing and *r* is the relative infection rate. A quadrat consisted of a 0.5 m length of row. Although a disease score was recorded for each quadrat, data were analysed on the basis of presence/absence of disease in each quadrat. This was considered to be a reasonable approach on the basis that the presence of any disease in a quadrat could potentially render the crop in that quadrat unmarketable or lead to returns. The value of *c* was fixed at 0.05 (the radius of the primary infection), and the model was specified with a binomial error distribution and logit link function. Parameter estimates are given in .

Table 3. Parameter estimates for model fitted to data from first coriander/bacterial blight spread trial.

Trial	Parameters		
	a	*b*	*r*
1 (Y2)	-11.8	-4.85	0.26
2 (Y2)	n/a*	n/a	n/a
3 (Y3)	-13.0	-3.39	0.27
4 (Y3)	-5.7	-1.8	0.04

* Estimation was not possible

The models had a good fit to the data as indicated by significant χ^2 values and visually (see Figure 2).



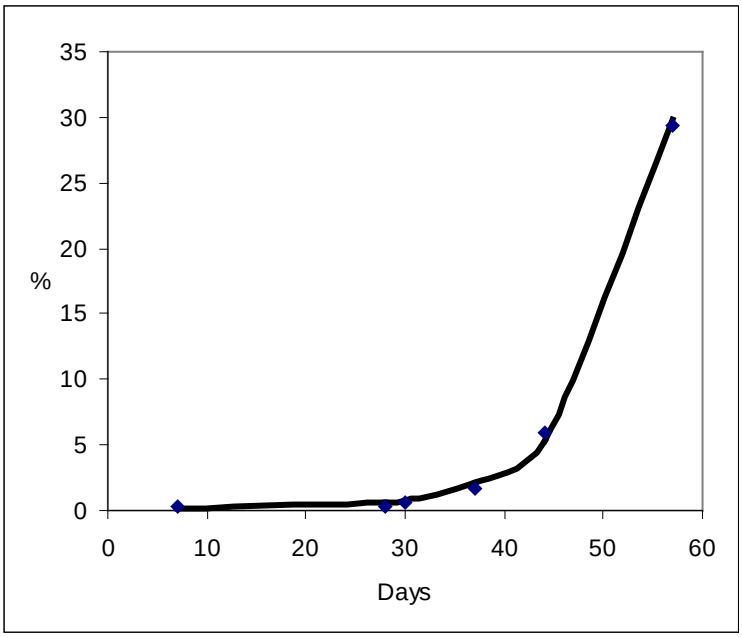


Figure 2. Change in the percentage of quadrats with bacterial blight symptoms in coriander spread trials 1 (top) and 3 (bottom) (10 m x 3 beds) with a single central point source of inoculum. The line represents the

Seed health standards

Some example scenarios used to develop recommendations for seed health standards are shown in . These show the predicted transmission, disease levels and expected seed test outcomes for a range of seed infection levels both in terms of the % of seeds infested and the numbers of bacteria on those infested seeds.

Table 4. Probability of transmission, and coriander bacterial blight disease incidence for an area of approx. 0.36 ha sown with 1 million seeds with infestation levels from 0.003% to 0.1% and bacterial numbers from 10^2 to 10^5 CFU per infested seed, together with the probability of detection in seed tests on either 1 x 3,000 seeds or 3 x 3,000 seeds.

Seed inf.			Prob.	Incidence	Pr +ve test[2]		Overall risk[3]	
1 in	% inf	CFU	Trans.[1]	Max %	1 x 3k	3 x 3k	1 x 3k	3 x 3k
30,000	0.003	1 x 10^2	0.019	19	0.03	0.08	0.02	0.02
30,000	0.003	1 x 10^3	0.037	19	0.09	0.25	0.03	0.03
30,000	0.003	1 x 10^4	0.069	19	0.10	0.26	0.06	0.05
30,000	0.003	1 x 10^5	**0.128**	19	0.10	0.26	**0.12**	0.09
15,000	0.007	1 x 10^2	0.038	33	0.06	0.16	0.04	0.03
15,000	0.007	1 x 10^3	0.072	33	0.18	0.44	0.06	0.04
15,000	0.007	1 x 10^4	**0.133**	33	0.18	0.45	**0.11**	0.07
15,000	0.007	1 x 10^5	**0.240**	33	0.18	0.45	**0.20**	**0.13**
10,000	0.010	1 x 10^2	0.057	45	0.08	0.23	0.05	0.04
10,000	0.010	1 x 10^3	**0.106**	45	0.25	0.58	0.08	0.04
10,000	0.010	1 x 10^4	**0.193**	45	0.26	0.59	**0.14**	0.08
10,000	0.010	1 x 10^5	**0.337**	45	0.26	0.59	**0.25**	**0.14**
5,000	0.020	1 x 10^2	**0.111**	70	0.16	0.40	0.09	0.07
5,000	0.020	1 x 10^3	**0.201**	70	0.44	0.82	**0.11**	0.04
5,000	0.020	1 x 10^4	**0.349**	70	0.45	0.83	**0.19**	0.06
5,000	0.020	1 x 10^5	**0.561**	70	0.45	0.83	**0.31**	0.09
1,000	0.100	1 x 10^2	**0.444**	95	0.57	0.92	**0.19**	0.03
1,000	0.100	1 x 10^3	**0.674**	95	0.94	1.00	0.04	0.00
1,000	0.100	1 x 10^4	**0.883**	95	0.95	1.00	0.04	0.00
1,000	0.100	1 x 10^5	**0.984**	95	0.95	1.00	0.05	0.00

[1] Probability of at least one infected seedling in the block. Values >0.1 in bold.
[2] Probability of detection in seed test performed according to PHS standard method.
[3] The overall risk is the probability of a negative test result combined with the probability of transmission. Shaded values are considered to represent an unacceptable risk.

Discussion

Seed testing

The seed test results indicated that a significant proportion of coriander seed lots may be infested with *Psc*. The levels of infestation were relatively high for a bacterial disease both in terms of the pathogen numbers and the high % of seeds infested. It is important to note that in the lots where *Psc* was not detected, due to sampling and the detection limits inherent in the test method, we cannot be certain that they are completely healthy therefore an upper 95% confidence limit is provided.

The seed test results could also bring into question the reliability of the test results from some seed testing laboratories which had previously performed tests on some of the lots and failed to detect the infestation despite the high levels present. This highlights the importance of ensuring that seed health testing laboratories have the appropriate experience, expertise and test methods for pathogen in question. Growers should not

assume that a particular laboratory has the expertise or methodology to perform a particular test.

Transmission

Transmission from seed to seedling is a fundamental pre-requisite for the development of disease in a crop and therefore quantifying this relationship is important information for defining seed health standards. To examine transmission we use a 'one-hit' theoretical model for infection, described by the equation:

$$p = 1 - e^{-wd}$$

where p is the probability of infection, d is the dose and w is the 'one-hit' probability. This model makes the assumption that each individual pathogen cell (or spore) is inherently capable of infection, but the probability of this occurring may be very small. The aim of the dose-response experiments was to estimate this 'one-hit' probability.

The transmission experiment in Year 1 used both naturally infested and artificially inoculated seed to look at dose/response relationships. Transmission occurred at a lower frequency than expected and was only detected at the highest inoculum level, providing an unreliable estimate. Therefore in order to obtain a more robust estimate, the transmission experiment was repeated using the two highest doses plus an additional dose. Transmission was detected in the two highest doses in this second experiment and the results combined with earlier data to provide an estimate of the one-hit transmission probability of 1.6×10^{-4} and a dose (scaling) parameter of 0.282. These values were then used to predict the likelihood of disease transmission for seed lots with different levels of infestation and examine these values in relation to the probability of detection for different seed health testing schemes (See).

Field Trials

Three of the four field trials to examine spread of coriander bacterial blight from a point source provided useful data and mathematical models were successfully fitted. A model was not fitted to the data in the other trial due to the unreliability of the data. The highest levels of disease and greatest spread were seen in Trial 3 (in Year 3) and this is reflected in the larger value for *r*, the relative infection rate, and smaller value for *b*, the disease gradient, compared to Trial 1. The second trial in Year 3 experienced relative dry conditions early on which limited disease development, and by the time spread first became apparent (in October) it had also become considerably cooler, limiting bacterial multiplication. This is then reflected in the relatively small value for *r* compared to the earlier sown trials. The location of some of the earliest observed symptoms in the trial, at the edge of the plot, neighbouring the location of the previously sown trial, suggested that these were the result of spread from crop debris from that previous trial. The occurrence of disease at the extreme edge would also contribute to the small value for *b* compared to the earlier sown trials.

Seed health standards

The example scenarios demonstrate the potential disease risks for a range seed lots and the overall risk for two testing schemes. Several other testing schemes (i.e. testing more or fewer seeds in total and for different sub-sample sizes) within the practical limitations of test method were considered but are not shown. When testing seed for bacterial pathogens the

variable which most affects the cost of testing is the number of sub-samples tested rather than the total number of seeds. Thus testing a single sample of 3,000 seeds is cheaper than testing 3 sub-samples of 1,000 seeds. On the other hand there is little/no difference in the cost of testing 3 sub-samples of 1,000 seeds compared to 3 sub-samples of 3,000 seeds. Although it would be tempting to test more seeds by increasing the (sub-) sample size, due to dilution effects this leads to reduced test sensitivity and increases the risk of detection failures for seed lots with high % infestation but low numbers of bacteria per infested seed. For routine testing of coriander seed for fresh leaf production in the field it is recommended to test at least 3 sub-samples of 3,000 seeds with a test sensitivity of 900 CFU, as this ensures that the overall risks for the different scenarios are mostly below 10%, which would seem to be an acceptable level. Testing a single sub-sample of 3,000 or 5,000 seeds would be cheaper option but clearly results in more scenarios where the overall risk is a more than 10%. It is important to bear in mind that these estimates are for a single crop derived from drilling 1 million seeds, for larger individual crops or for multiple crops drilled in sequence across a field (which can be considered as a single crop over a larger area) the risks will be greater, most noticeably when testing just a single sub-sample. For seed or spice crops which are in the field for much longer (so that the maximum potential disease incidence will be greater for a given seed infestation level) growers and seed producers should consider applying a more stringent seed health standard.

Alternative seed treatment methods for control of coriander bacterial blight

Introduction

There are currently no plant protection products approved for the control of *Psc* in coriander, and there are no products approved for seed treatment.

The relevant literature was searched and evaluated to identify potential seed treatments, particularly those that might provide alternatives to conventional fungicidal seed treatments, for testing in project year 2.

Taylor (1980) reduced seed infection by the use of slurry treatments with antibiotics, but due to concerns about the use of antibiotics these are unlikely to ever receive approval in the EC, and were not considered worth pursuing further.

Work done in Australia (Dennis and Wilson 1997; Hooper and Dennis 2002) suggested that treatment of coriander with dilute HCl (hydrochloric acid) for 24 hours followed by washing and drying of the seed can be effective in reducing levels of *Psc* and consequent improvement of yield. This approach has a number of practical problems: handling of a hazardous substance, the long soak time and significant drying time (5 days). Given these practical difficulties and the fact that it was not always completely effective, with some bacteria surviving in some seed lots, this approach was not considered to be worth pursuing further.

In a recent EC-funded project (STOVE) to examine organically acceptable treatments for a number of vegetable crop/pathogen combinations, both hot water and hot humidified air (aerated steam) were found to be effective on a wide range of crops and pathogens (both bacterial and fungal). The main problem with routine application of both these treatments was the need to optimise on a lot by lot basis for maximum efficacy, although when dealing with large quantities of high value seed this is perhaps not such a great issue.

In comparing the two treatments the hot air treatment has the major advantage that the seed does not require drying after treatment, however the capital investment and licensing requirements mean that start-up costs may be higher for hot air. Unfortunately facilities were not available for testing hot air treatment within the scope of this project.

The STOVE project also indicated two biological control agents and a natural product that may have some value in controlling seed-borne bacterial diseases. The *Bacillus subtilis* based products Serenade™ and Subtilex™, and the natural product thyme oil, all had *in vitro* anti-bacterial activity.

Broad spectrum disinfectants/biocides are often considered as potential seed treatments for control of seed-borne bacterial diseases. Sodium hypochlorite (bleach) and peroxyacetic acid (e.g. Jet 5) both currently have pesticide approval as Commodity Substances, and it is therefore often assumed that they can be used as seed treatments. The situation has been clarified with the Pesticides Safety Directorate (PSD) and it is clear that Commodity Substances can only be used for the crops/situations specifically mentioned in the approval, therefore their use as seed treatments is ILLEGAL, unless or until such time as a specific approval is obtained. This does not mean their potential as seed treatment should not be

investigated, but it should be made clear that pesticide approval would be required before their legal use could be permitted. Chlorine dioxide does not have approval but is increasingly being used as an alternative to chlorine/hypochlorite, especially for salad washing. It is often considered to be more effective than chlorine, is less corrosive and its biocidal activity is not affected by pH.

The potential of several of these seed treatments was examined experimentally. These included: hot water, thyme oil (white, Sigma), two biological control agents (BCAs) (Subtilex™ and Serenade Max™, both are strains of *Bacillus subtilis*) and one conventional disinfectant (chlorine dioxide).

Materials and Methods

Seed

Larger quantities of the seed lots with high levels of seed infestation identified in Year 1 were obtained from seed companies. In initial studies physical/chemical treatment studies (E895) seed lot S1045 was used. But following an apparent decline in infestation levels seed lot S1072 was used. In addition, BCAs were also evaluated using an artificially inoculated seed lot (S1081).

Seed Treatment

Hot water treatment was initially done in glass beakers within a thermostatically controlled water bath. Each treatment was done in a separate beaker to avoid the potential for cross-contamination. Beakers (600 ml) containing approx. 250 ml of distilled water were allowed to equilibrate with the temperature of the surrounding water bath. An aliquot of seeds was then transferred to the beaker. As coriander seed floats, seed was submerged in the water by placing a 250 ml conical flask, with a diameter just slightly less that the internal diameter of the beaker, and containing 100 ml of water also at the same temperature as the water bath on top of the seeds. Due to the immediate drop in temperature that occurs in the beaker when the seed is introduced, the water bath and initial temperature of the water in the beaker and flask were maintained 2-3°C above the target temperature. The actual temperature that the seed was exposed to was checked with a thermometer at 5 min intervals during the course of treatment.

Later hot water treatments were done using specially fabricated stainless steel mesh baskets (2 mm hole size) with closed lids. These ensured rapid and complete immersion of seed and enabled agitation to ensure more rapid achievement of the target temperatures. Baskets containing seeds were immersed directly in the water bath (held at the target temperature) for the requisite time, seeds were then allowed to drain and were dried in the airflow of a fan whilst remaining in the mesh baskets.

Thyme oil (white FCC, Sigma W306509) treatments were done with stabilised oil/water emulsions at concentrations from 1 to 10%. Aliquots of seed were placed into a suitably sized conical flask and an excess of oil/water emulsion added, the flask was then shaken to mix and left to stand for 30 min at room temperature (RT).

Chlorine dioxide treatment was done using sachets of a commercially available chlorine dioxide generator (Tristel Fusion). Treatment was done with two different concentrations

(100 and 500 ppm). The sachets contain two separate components, which when mixed by squeezing give a stock solution containing approx. 5,000 ppm; this stock solution was then diluted to obtain the required concentrations for treatment. Seed was treated in a similar way to the hot water treatment, with aliquots of seed added to a beaker containing the chlorine dioxide solution and submerged by the use of a conical flask containing distilled water. Seed was immersed in the solutions for 30 min.

For all except the later hot water treatments, once the treatment time had elapsed, seed was separated from the treatment liquid by pouring through a suitable sieve, the seed in the sieve was then blotted with paper towels to remove excess liquid and then tipped into a plastic container and allowed to dry for 2 d at RT under the airflow of a fan. Following drying, the treated seed was packaged into 'seal-easy' polythene bags and stored in the refrigerator until testing.

Treatment of the seed with BCAs (Subtilex and Serenade Max, *Bacillus subtilis* strains) which are formulated as dry powders, was done by adding an appropriate amount of the product to an aliquot of seed in a polythene bag at the rate of 20 mg of product per gram of seed, then shaken to mix thoroughly until the seed was visibly and evenly coated.

Germination

Germination was tested according to the methods described in the International Rules for Seed Testing (ISTA 2007) using the 'BP' (Between Paper) method.

Evaluation of physical/chemical treatments

The efficacy of the physical/chemical treatments was evaluated by testing several sub-samples of the treated seeds, following the standard method described previously.

Evaluation of biological treatments

As biological treatments would not be expected to have direct effects on dry seed, it is inappropriate to evaluate their efficacy by direct testing of the seed. The efficacies of the BCAs were therefore examined by testing their effect on disease/pathogen transmission from seed to seedling. The earlier transmission experiments had indicated a relatively low one-hit transmission probability, therefore the approach for these transmission experiments was modified from that used previously to facilitate more accurate estimation of lower transmission rates.

Standard seed trays (approx 30 x 20 cm) were loosely filled with compost (Bullrush modular organic) and lightly compressed so that the surface of the compost was approx. 1 cm below the rim. Aliquots of approx. 200 seeds (by weight) were scattered over the surface of each tray and then covered with compost. Ten seed trays (i.e. total of 2000 fruits) were used for each treatment. Trays were set out on the glasshouse bench in blocks of 5 trays (i.e. 2 blocks per treatment) and watered by means of an overhead sprinkler system. After initial watering in, trays were watered daily at 0800 for 3 min. The glasshouse temperature regime was set to a min. of 18/15^{o}C day/night and venting at 20/20^{o}C day/night. Supplemental lighting was provided to ensure a min. day length of 12 h.

Plants were observed for the presence of symptoms at regular intervals. When found the numbers of plants with symptoms was recorded in each tray.

Rather than relying on symptom expression, transmission was estimated by determining the proportion of seedlings contaminated with the pathogen (*Psc*), as in the earlier transmission experiments. Approx. one month after sowing, when plants had 2-3 true leaves, a sample of 50 plants was collected from each tray in each treatment. Samples were collected by cutting the stems with scissors just below the cotyledons and were placed directly into new stomacher bags. Within a tray, samples were collected systematically to ensure coverage of the whole tray. To minimise the potential for cross contamination, scissors and hands were disinfected between each treatment using 70% iso-propanol.

Samples were processed as described for transmission in Year 1.

Statistical analyses

For the physical/chemical treatments the proportions of infested seeds and mean numbers of bacteria on seeds following treatment were estimated as described previously.

For the BCAs, transmission was estimated using STPro™ seed test analysis program. Estimates were obtained on the basis of entire trays, i.e. a positive result for a sample of plants from a single tray was taken to indicate that there was at least one transmission event in that tray (sown with 200 seeds (fruits)). Bacterial numbers were estimated by fitting a Generalised Linear Model using the Genstat statistical analysis program (Payne *et al.* 2005).

Results

Physical/chemical treatments

In initial tests, using naturally infested seed lot S1045, *Psc* was not detected in the untreated control samples, thereby obviating evaluation of efficacy any of the treatments; nevertheless some data was obtained on the effects of the hot water treatments on germination (see). Further testing/re-testing of stocks of infested seed lots was therefore necessary to identify an alternative seed lot for use in the treatment studies, and subsequently evaluation was done with seed lot S1072.

The total % germination following treatment is shown in . Due to the nature of coriander fruits and the potential adverse affect of one seed in a fruit on the other, these germination tests were difficult to evaluate and interpret, especially with respect to the proportion of normal/abnormal seedlings (data not shown). Nevertheless they do indicate a trend to improved germination following most treatments, with possible detrimental effects only for the most stringent (highest temp./longest duration) hot water treatment.

Table 5. Germination in two coriander seed lots following hot water/chemical treatments

Treatment[1]	S1045	S1072	
	%Seed[2]	%Fruits[3]	%Seed[2]
Untreated	43.3	48.3	31.7
ClO_2 (100 ppm)	nt	60.0	57.5
ClO_2 (50 ppm)	nt	81.7	58.3
HW 50°C 15 min	37.5	70.0	43.3
HW 50°C 30 min	65.0	68.3	45.0
HW 53°C 15 min	51.7	nt	nt
HW 53°C 30 min	67.5	nt	nt
HW 55°C 15 min	80.0	70.0	43.3
HW 55°C 30 min	44.2	20.0	10.8
Thyme oil (10%)	nt	45.0	27.5

[1] ClO_2 = Chlorine dioxide; HW = Hot water
[2] Assuming two seeds per fruit.
[3] One or more seedlings per fruit.

Post-treatment seed test results for initial tests with naturally infested seed lot S1072 are summarised in and Figure 3. It should be noted that where all sub-samples tested were positive (e.g. untreated) or negative (e.g. HW55-30) only a lower or upper confidence limit for the % infested can be estimated. *Psc* was not detected in any sub-samples following treatment with hot water at 55°C for 15 or 30 min. Hot water at 50°C also gave significant reductions in both the % seed infested and numbers of bacteria; smaller, but also significant reductions were obtained with Thyme oil treatment. Both chlorine dioxide treatments failed to give any useful reductions.

Table 6. Summary of seed tests on bacterial blight infested coriander seed (seed lot S1072) following hot water/chemical treatment.

Treatment[1]	% Infested[2]			Log_{10}(Bacteria)[3]	
	Estimate	Lower	Upper	Estimate	s.e.
Untreated	>0.93	0.93	100	4.20	0.06
ClO_2 (100 ppm)	>0.18	0.18	100	4.03	0.20
ClO_2 (50 ppm)	>0.18	0.18	100	3.23	0.43
HW 50°C 15 min	1.1	0.22	3.7	-0.43	2.96
HW 50°C 30 min	0.029	0.002	0.14	0.30	0.62
HW 55°C 15 min	<0.067	0	0.067	-	-
HW 55°C 30 min	<0.067	0	0.067	-	-
Thyme oil (10%)	2.2	0.39	7.3	1.49	0.91

[1] ClO_2 = Chlorine dioxide; HW = Hot water
[2] % infested and lower and upper 95% confidence limits estimated from multiple seed tests using STPro™.
[3] Log_{10}(Numbers of bacteria per seed) are a weighted mean obtained as predictions from a GLM in Genstat, together with approximate standard errors.

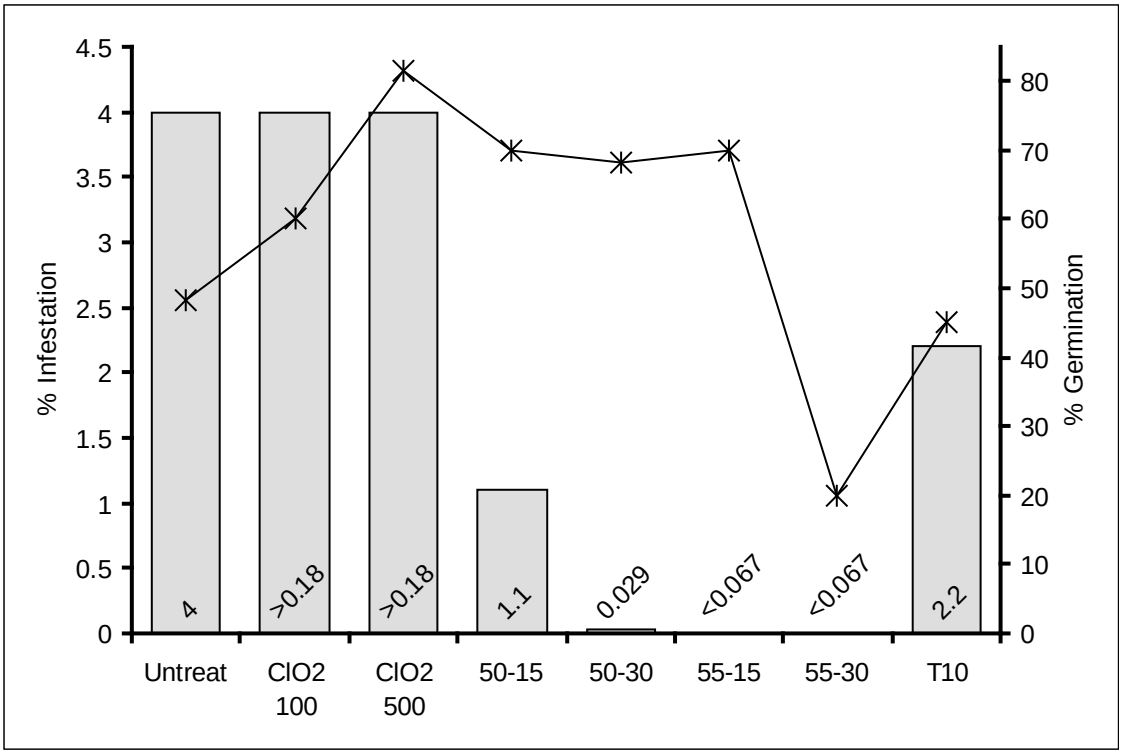


Figure 3. Effect of initial seed treatments with chlorine dioxide (100 and 500 ppm), hot water (50°C for 15 and 30 min, 55°C for 15 and 30 min) and thyme oil (10%) on level of infestation with *Pseudomonas syringae* pv. *coriandricola* in naturally infested coriander seed lot S1072.

Subsequently, two hot water treatments were examined on six seed lots with a range of infestation levels. Results are shown in Figure 4. In five of the six seed lots the *Psc* was reduced to undetectable levels by both temperature/time combinations. The detection limits achieved in these tests was commensurate with the level proposed as the seed health standard for field crops (see above). Apparent eradication was not achieved for one highly

infested seed lot by either temperature/time combination, but in this case a greater reduction was achieved by the longer time at a lower temperature (53°C for 30 min). For some seed lots treatment at 53°C for 30 min reduced germination compared to untreated seed, in these lots reducing the temperature slightly to 52°C improved germination but was still able to apparently eradicate the pathogen.

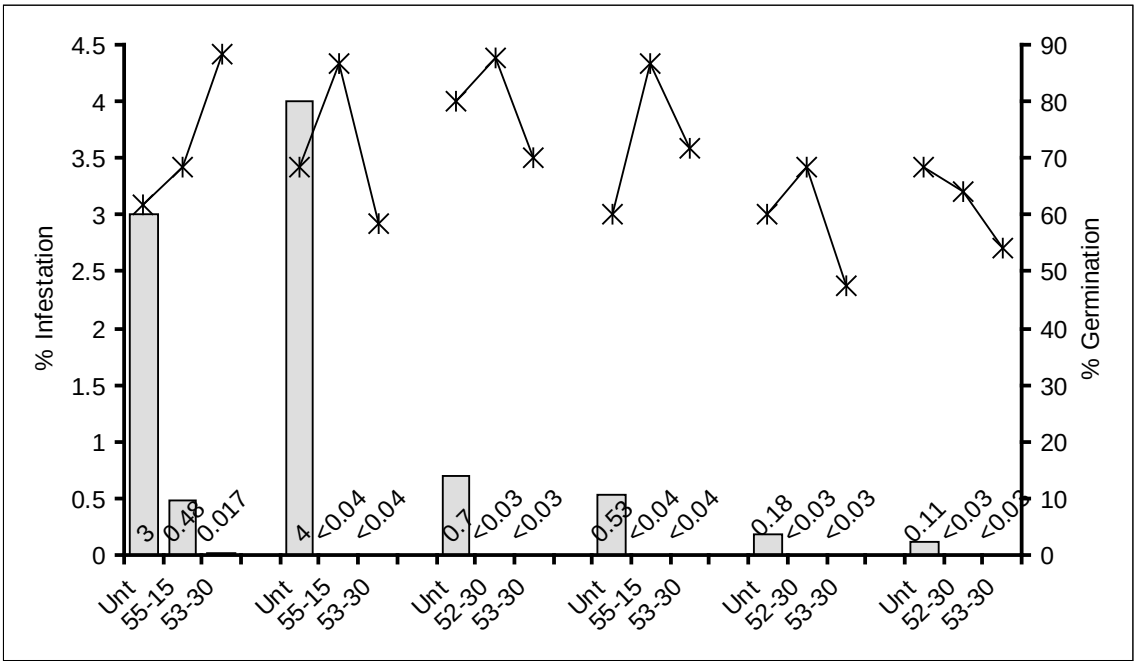


Figure 4. Effect of hot water treatment on germination and infestation levels in six coriander seed lots, naturally infested with *Pseudomonas syringae* pv. *coriandricola*. Bars represent the infestation level, lines represent germination. Unt = untreated; 55-15 = 55°C for 15 min; 53-30 = 53°C for 30 min; etc.

Additional tests with thyme oil were also done on three additional seed lots. At the highest concentrations (10%) although *Psc* was apparently eradicated, there was a marked and unacceptable reduction in germination. At lower concentrations (1 and 5%) the impact on germination was less but the reduction in infestation levels achieved was also lower.

Biologicals

Results of the transmission studies in seed treated with BCAs are shown in and Figure 5.

Table 7. Summary of transmission studies on two bacterial blight infested coriander seed lots treated with BCAs

Treatment	Symps[1]	No. trays[2]	%Transmission[3]			$Log_{10(}$(Bacteria)[4]	
			Estimate	Lower	Upper	Estimate	s.e
Inoculated (S1081)							
Untreated	6	10	>0.67	0.67	100	5.99	0.05
Subtilex	8	10	>0.67	0.67	100	5.79	0.06
Serenade	2	10	>0.67	0.67	100	5.64	0.07
Nat. Inf. (S1072)							
Untreated	0	3	0.18	0.04	0.47	1.28	0.89
Subtilex	0	1	0.05	0.003	0.23	1.02	1.15
Serenade	0	0	<0.15	0	0.15	-	-

[1] Total number of plants with visible symptoms in all ten trays.

[2] Number of trays in which *Psc* was detected by 'leaf washings'.

[3] % transmission and lower and upper confidence limits estimated using STPro™, assuming each sample represents the whole tray of 200 seeds.

[4] $Log_{10(}$(Numbers of bacteria per plant) are a weighted mean obtained as predictions from a GLM in Genstat, together with approximate standard errors

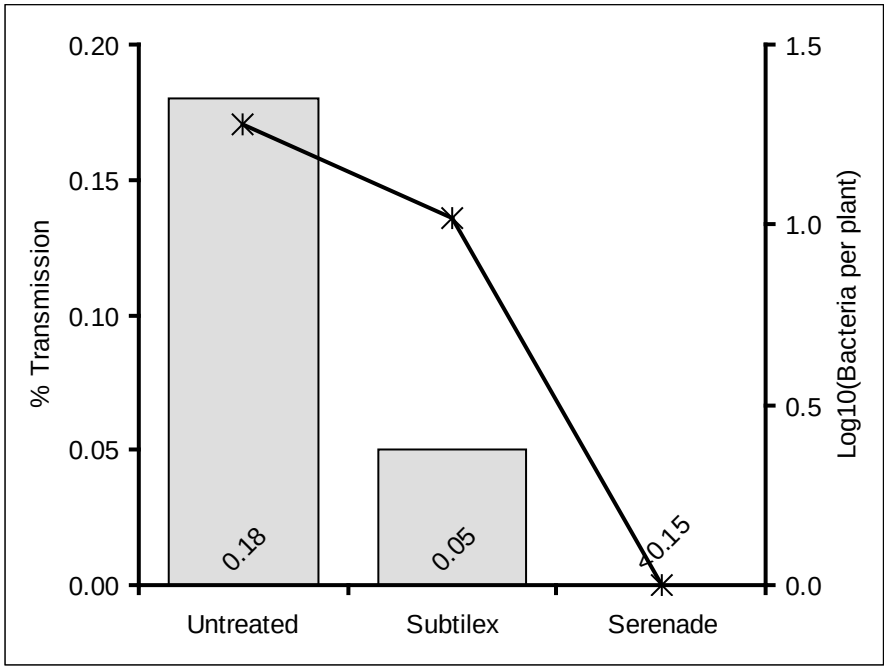


Figure 5. Effect of treatment with biologicals on the seed to seedling transmission of *Pseudomonas syringae* pv. *coriandricola* for a naturally infested coriander seed lot.

Inoculated seed. In the inoculated seed lot, some symptoms were observed in a few trays in each treatment at around 21 d after sowing, but given the small number observed and the difficulty of seeing them, no conclusions should be drawn from these values. Leaf washings

at 31 d indicated the presence of *Psc* in all trays of all treatments, thus no. comparisons of transmission were possible for the inoculated seed lot.

Naturally infested seed. No symptoms were observed in any trays from any treatment during the course of the experiment. Leaf washings at 32 d indicated the presence of *Psc* in three trays grown from untreated seed, one tray grown from Subtilex treated seed and no trays grown from Serenade treated seed.

An analysis of deviance of the numbers of bacteria (both experiments combined) indicated a significant effect of treatment, with significant reductions in the mean number of bacteria per plant for both Subtilex and Serenade Max treated seed.

Discussion

Results for initial tests using seed lot S1045 were problematical, as the pathogen was not detected in the untreated control sample, therefore no conclusions could be drawn about the efficacy of treatments for this seed lot.

Seed lot S1045 had been selected for treatment work because seed tests in year 1 had indicated a relatively high level of infestation with *Psc* (4.4%) and a large batch was available. Further testing of the second batch indicated that, whilst it was still contaminated, the estimated infestation level was much lower than in year 1. There are a two possible explanations for this difference:

1. The seed lot was heterogeneous with respect to *Psc* infestation, and sampling was inadequate. By definition a seed lot should be homogenous and well mixed. It is possible that there were 'hot spots' of *Psc* infestation within the bulk and combined with inadequate sampling meant that the initial sample was drawn from such a 'hot-spot'.
2. Levels of seed infestation/populations of *Psc* on the seed had declined during the year between samples being drawn.

Given that results obtained for other seed lots indicated no major declines in infestation over a similar period, it seems most likely that the differences in results are mostly likely to be explained by (1). This highlights the vital importance of adequate primary sampling when drawing samples from large bulks of seeds.

The initial results of seed tests on physically/chemically treated coriander seeds suggested that hot water was the most promising treatment and was worthy of more detailed investigation of treatment parameters, and with more seed lots. All hot water treatments gave very significant reductions in *Psc*. Initial testing suggested that the best treatment for routine use, giving the most reliable reductions in *Psc was*, 53°C for 30 min, without any reduction in germination. However, when tested on a wider range of seed lots, some seed lots suffered a reduction in germination; reducing the temperature to 52°C in these seed lots preserved germination whilst still reducing *Psc* to undetectable levels. Except for one seed lot, the reductions achieved by 53 or 52°C for 30 min would be sufficient for the seed lots to achieve the proposed seed health standard.

In initial tests, Thyme oil at 10% also gave a significant reductions in *Psc*, albeit with an adverse effect on germination. Subsequently it was tested at lower concentrations, these had less impact on germination, but also had less impact on *Psc,* given the apparent success of thyme oil for other host/pathogen combinations it could be worth pursuing alternative formulations and treatment durations.

In the initial tests chlorine dioxide at the concentrations used (100 and 500 ppm) appeared to have no effect on the levels of *Psc* infestation, given the promise shown by the other treatments, it was not pursued further.

Because of the presumed ways in which the BCAs work, seed testing cannot be used to test their efficacy. The two BCAs (Subtilex and Serenade Max) were therefore initially evaluated in glasshouse transmission experiments using both inoculated and naturally infected seed lots. This requires a lot more effort than seed testing and limits the number of experimental units and total numbers of seeds which can be examined and hence the 'statistical power' of the data analysis. Nevertheless clear indications of reductions in transmission and bacterial populations were obtained for both BCAs. This is also consistent with the results obtained in FV 335 (Roberts 2009) for another bacterial disease (black rot of Brassicas). Given the promise shown by the other treatments and apparent lack of interest in the manufacturer of Serenade in pursuing a registration for seed treatment, it was decided to focus efforts on the other treatments. [Note since this work was completed, the manufacturer of Serenade has shown a renewed interest in pursuing registration as a seed treatment].

Developing appropriate seed health standards for parsley

Introduction

As parsley Septoria is seed-borne an important means of control is through the use of 'clean' seed. There are currently no defined seed health standards for parsley and no officially used seed testing methods. The aim of this part of the work was to further investigate the seed-borne nature of *Septoria petroselini* on parsley as a basis for developing seed health standards. Initially, seed testing was done using commercial seed lots to determine:

- Percentage incidence of seeds with pycnidia
- Percentage incidence of seeds that gave conidial release from pycnidia and subsequent germination
- Mean numbers of conidia per seed, using seed with and without pycnidia

As for coriander bacterial blight, the objective was to then estimate the rate of transmission from seed to seedling in dose-response experiments and to estimate the rate of disease spread.

Materials and methods

Seed testing

In project year 1, seven separate seed lots were used that were supplied by two commercial seed houses and reported to be infected with *Septoria petroselini.*

For each seed lot, a 20 g composite sample (using the 'spoon method'; Mathur and Kongsdal, 2003) was taken. From the sample, four sub-samples of 100 seed were taken at random (using the 'hand-halving method'; Mathur and Kongsdal, 2003). For each sub-sample, the percentage of seeds with visible pycnidia was determined using a low power microscope. The four sub-samples of 100 seed were plated on potato dextrose agar amended with streptomycin (PDA+S), with each seed in a droplet of sterile distilled water (SDW) (25 seeds per plate, 16 plates in total). The seeds in water droplets were checked immediately for the incidence of spore release, then incubated at 20°C for 16-20 h. The seeds were re-examined to determine the incidence of seeds with spore release, and the incidence of seeds with spore release and spore germination.

For each of the seven seed lots, four sub-samples of 100 seeds with pycnidia and four sub-samples of 400 seeds without pycnidia were collected. Each sub-sample was soaked separately in 5 ml sterile distilled water for 1 h. For each lot, after agitating the seed mixture, the liquid was decanted through sterile muslin into a Universal tube and centrifuged at 4000 rpm for 30 min. The supernatant was discarded and the pellet re-suspended in 1 ml sterile distilled water. For each seed lot (four sub-samples with pycnidia, and four without pycnidia), the concentration of spores/ml was calculated using a haemocytometer and microscope.

Seed transmission

The frequency of pathogen transmission was studied in year 1 using seven seed lots supplied by two commercial seed houses and reported to be infected with *Septoria*

petroselini. For each seed lot, a composite sample of 50 g was taken using the spoon method of Mathur and Kongsdal (2003). Parsley seed from the sample was sown in six modified module trays (204 modules per tray) in F1 compost (Scotts), with approximately three seeds per module (modified to four trays of 294 modules each for seed batch G). The compost surface was covered with a fine layer of vermiculite. An extra full tray was sown (approximately 3 seeds per module) to use as spare modules.

The module trays were placed on damp capillary matting in a ventilated glasshouse. The compost was kept sufficiently moist to enable seed germination, without water-logging. There was no overhead watering once seedlings had emerged. The trays were maintained in a glasshouse until emergence in at least 50% of modules of each tray was achieved (approximately 3 weeks). Where necessary, modules from the spare tray were used to replace empty modules, to give approximately even numbers of emerged modules in each tray.

The trays were examined to confirm that seedlings were free of Septoria lesions at the time of transfer to two controlled environment (CE) cabinets (24°C/18°C, 16 h day/8 h night, 75% RH) which each contained a misting unit. The six module trays containing parsley seedlings were placed in the cabinets, three trays per cabinets. The plants were misted (1.2 L/h) for 72 h to maintain continuous leaf wetness. Subsequently, the trays were hand-watered as necessary (carefully to avoid splash) in order to maintain moist but not water-logged compost.

From 7-18 days after seedling transfer to the CE cabinets, the trays were examined at least twice per week for symptom development due to *S. petroselini*. For each tray, the number and position of modules with Septoria lesions was recorded and symptoms confirmed microscopically. Infected seedlings were removed from the tray once symptoms have been confirmed and recorded.

The conditions in the cabinets were selected to favour the development of parsley Septoria, based on results from Krauthausen *et al*., 2001, and Kurt and Tok, 2006. To ensure that lesion development on seedlings related to primary seed-borne infection as opposed to secondary disease development, conditions favourable to disease development were applied as soon as seedlings had emerged. The trays were monitored initially for 20 days only to ensure that disease incidence related to a primary rather than secondary disease cycles (minimum time to primary symptom development is approximately 10 days, plus 10 days for development of secondary symptoms, based on Krauthausen *et al*., 2001). If there was no symptom development within this time, the trays were monitored weekly for a further 4 weeks.

Standard deviations were calculated for results on percentage seeds with visible pycnidia, and percentage seeds showing spore release and spore germination. Confidence limits (95%) were calculated for the number of spores per seed, for parsley seed with and without pycnidia. To examine the probability of transmission of *S. petroselini* from parsley seed to seedlings, a ‘one-hit’ theoretical model for infection was used, following a similar approach as described above for *Psc* on coriander seed.

Seed transmission using artificially infested seed

Because of limited data on pathogen transmission using naturally infested seed, an experiment was done in Project year 3 to determine transmission rates using seed that had been artificially infested with S. *petroselini.*

Using a commercial parsley seed lot, 1000 seed weight was determined, then ten composite samples of 5 g were prepared using the 'spoon method' of Mathur and Kongsdal (2003). Each 5 g sample was placed in a clean Petri dish labelled with treatment and replicate number (one replicate for treatment 1, three replicates for treatments 2-4).Treatments were as follows:

1. Uninfested control seed (no treatment)

2. Seed rolled in a single culture of sporulating *S. petroselini* (5 min)

3. Seed rolled successively in two cultures of sporulating *S. petroselini* (5 min x 2)

4. Seed rolled successively in three cultures of sporulating *S. petroselini* (5 min x 3)

For the inoculation procedure, seed samples were placed in a Petri dish containing a sporulating pure culture of *S. petroselini* on V8 agar. After replacing the lid, the dish was shaken to ensure all seed was in contact with the culture surface. In addition a blunt sterile spatula was used to roll the seed across the culture surface. This process was continued for 5 min. For treatment 3, the seed was transferred to a 2nd fresh culture for a further 5 min. For treatment 4, the seed was transferred to a 2nd, then a 3rd fresh culture, each for 5 min.

After each inoculation treatment had been completed, a 1 g sub-sample was taken from each replicate and placed in a Universal tube. The remaining seed for each treatment was combined.

At 3 h after inoculation, for each 1 g sub-sample, 3 ml sterile SDW was placed in the Universal tube. The tubes were left for 30 min then agitated for 30 sec. The spore concentration was determined using haemocytometer and microscope, then used to estimate spore loading per seed. For each sub-sample, 100 ul of the spore suspension was streaked onto a plate of PDA+S. The plates were incubated for 16 h at 20^{o}C then percentage spore germination determined by viewing counting numbers of spores germinated for 3 lots of 100 spores, using a microscope (x 400).

For each treatment, parsley seed was sown in four 308 module trays in F1 compost (Scotts) (1232 modules per treatment) with one seed per module at a depth of approximately 5 mm. The compost surface was covered with a fine layer of vermiculite. An extra half tray was sown for each treatment (as spares). The modules were placed on damp capillary matting in a ventilated glasshouse. There was no overhead watering during seedling germination and emergence. Trays for each treatment were placed together and separated from other treatments on the bench. The trays were maintained at approx 20^{o}C until at least 50% emergence had occurred; non-emerged seedlings were replaced with spare modules from the correct treatment. After 14 days, the trays were misted and covered with a polythene tent to ensure continuous leaf wetness for 72 h at 20-23^{o}C. This part of the experiment was done in a glasshouse rather than the CE cabinets (used previously for naturally infested seed),

because of space requirements. After the period of continuous leaf wetness, the trays were misted and examined daily for symptom development for approximately 6 weeks.

Disease spread

The experiment was sited in a polytunnel at ADAS Arthur Rickwood, Cambs. The crop used was of a curly leaf parsley variety (Bravour) that had been planted using parsley seedlings from modules in November 2008. The crop was planted almost continuously along the row (3 cm spacing) with 20 cm between rows, giving an area of 21 rows and 7 m length. The crop was overwintered then cut back to stem bases. One day after crop cutting (4 June 2009), a potted plant of parsley var. Bravour with typical symptoms of Septoria was placed as an infector plant in the centre of the experimental area. To provide conditions that were conducive for disease development, overhead irrigation was applied for 10 min daily for the next 8 weeks. The trial area was monitored regularly for development of parsley Septoria symptoms, by determining the number of plants infected in quadrats of 3 rows wide (0.6 m) by 0.5 m length.

Results

Seed testing

When seeds were examined microscopically, pycnidia of *S. petroselini* were visible on seed from all seven batches tested, with one batch containing 40% of seeds with pycnidia (Table 8; Appendix 3). Spore release from pycnidia was observed for five out of the seven seed lots (no release for lots E and F). However, subsequent germination of released spores (indicating pathogen viability) was observed for only two out of the seven lots (lots D and G).

Table 8. Characterisation of parsley seed lots to determine infection levels of *Septoria petroselini*

Seed characteristic	Parsley seed lot code						
	A	B	C	D	E	F	G
% seed with visible pycnidia*	40.3	34.3	0.5	6.3	2.0	0.5	11.0
% seed with spore release at 0 h**	1.5	13.5	0.3	1.8	0.0	0.0	2.0
% seed with spore release at 24 h**	9.8	31.0	0.3	5.0	0.0	0.0	7.8
% seed with spores germinating after 24 h*	0.0	0.0	0.0	0.3	0.0	0.0	2.0

* no. of seeds examined was 400
** no. of seeds examined was 100 (or 200 for batches A and B)

Examination of washings from all seven seed lots showed that spores of *S. petroselini* can be present on seeds both with and without visible pycnidia (Table 9). For all seed lots (except lot C for which few seeds with pycnidia could be found), spore numbers for seeds with pycnidia were equal to or greater than for seeds without pycnidia.

Seed transmission

For seed lots A-F, there was no development of Septoria on the parsley seedlings grown in module trays and incubated under conditions conducive for development of Septoria leaf

spot. These results confirm that there was no transmission of *S. petroselini* from seed to seedling for these seed lots, despite the presence of pycnidia on seed.

For seed lot G, there was no symptom development within the initial 20 days of monitoring. However, lesions subsequently developed and were assessed 45 days after seedlings were initially placed in the CE cabinets. The presence of lesions on the plants confirmed that for parsley seed lot G, transmission of *S. petroselini* had occurred from seed to seedlings. The incidence and distribution of seedlings with Septoria lesions is shown in Appendix 3 for a single tray (similar results were observed for the other trays; data not presented). Estimates of the one-hit probability of transmission (probability of transmission of one spore on one seed) varied, with values of 9.0×10^{-5}, 8.7×10^{-5} or 6.2×10^{-5}, depending on whether primary foci of infection were considered at the seedling, cell or cluster level, respectively. This estimate assumed that transmitted spores could have come from seed with or without pycnidia, with dose rate weighted according to the proportion of seeds with pycnidia. Interestingly, the one-hit probability of infection appears lower if it is assumed that only seed with pycnidia can contribute to transmission (4.6×10^{-5}, 4.4×10^{-5} or 3.0×10^{-5} depending on whether primary foci of infection were considered at the seedling, cell or cluster level, respectively).

Table 9. Spore counts (*Septoria petroselini*) from parsley seeds with or without pycnidia

Seed Lot	+/- pycnidia	No. seeds per rep	Mean no. of spores per seed**	95% confidence limits	
				Lower	Upper
A	+	200	175.0	28.0	322.0
	-	400	37.0	13.0	62.0
B	+	200	5950.0	4460.1	7439.4
	-	400	400.0	188.3	611.7
C*	+	13-27	0.0	0.0	0.0
	-	400	9.4	-9.0	27.8
D	+	100	150.0	30.0	270.0
	-	400	150.0	53.4	246.7
E	+	100	37.5	-36.0	111.0
	-	400	18.8	-2.5	40.0
F	+	100	150.0	30.0	270.0
	-	400	46.9	0.6	93.1
G	+	100	862.5	677.5	1047.5
	-	400	28.1	-7.1	63.3

* Few seeds with pycnidia found
**Mean of 4 reps

Seed transmission using artificially infested seed

The method used was successful in applying viable spores of *S. petroselini* to parsley seed (Table 10). This confirmed results from a preliminary experiment using a different parsley seed lot (data not presented), in which comparable numbers of viable spores were present on parsley seed at 3 h and also 24 h after artificial infestation. The infestation method provided different levels of spore loading, although extended treatment with *S. petroselini* in treatment 4, did not result in a higher loading than treatment 3.

Table 10. Spore loading and germination, 3 h after artificial infestation of parsley seed with *S. petroselini*

Treatment with *S. petroselini*	Mean spores per seed	Standard deviation	Mean % spore germination
1. Untreated	0	-	0
2. 1 x 5 min	85,139	34,958	57
3. 2 x 5 min	147,059	44,597	89
4. 3 x 5 min	130,450	65,139	65

Despite the presence of viable spores on parsley at the time of sowing and provision of environmental conditions conducive for disease development, there was no development of Septoria lesions on parsley seedlings even at 8 weeks after sowing. Other than reducing seed numbers per module (which could have altered micro-climate), other conditions of leaf wetness and temperature were comparable to those in the first experiment using naturally infested seed.

Disease spread

Symptoms of Septoria were first observed 12 d after placing the infector plant in the experimental area. For the first 2 weeks after introduction of the infector plant, disease

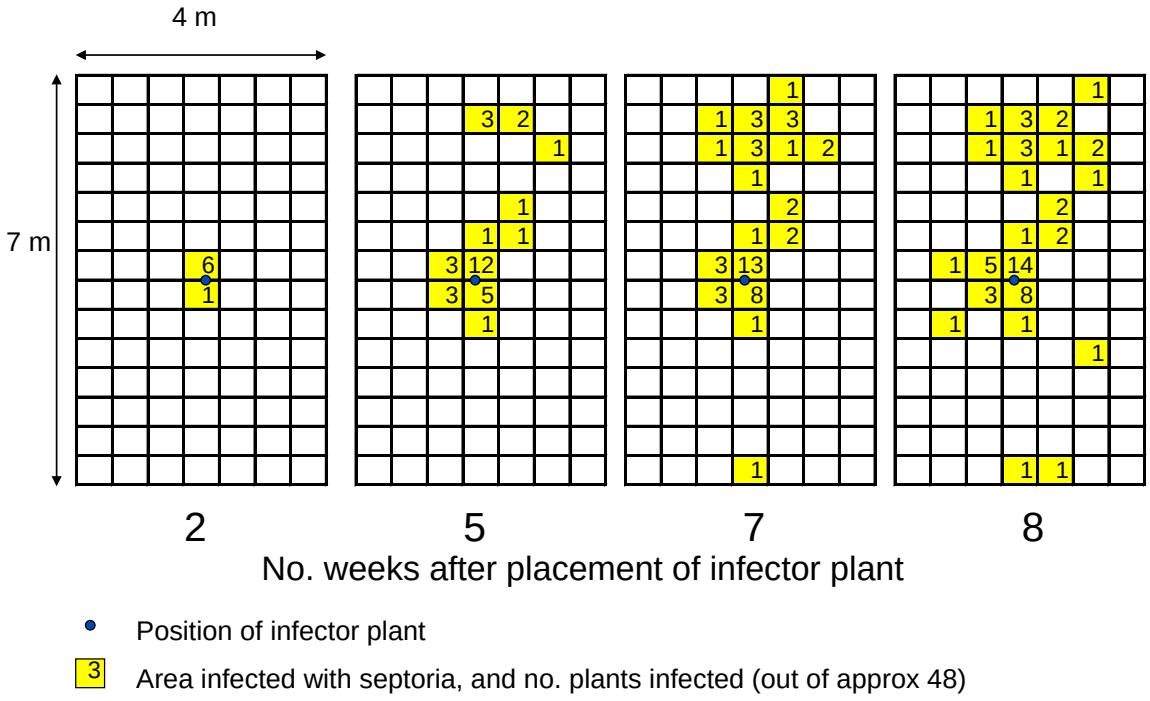


Figure 6. Spread of septoria from a single infection focus on parsley, Cambs 2009

symptoms all occurred less than 0.5 m from the infection point. By 5 weeks, symptoms had spread up to 3 m along the length of the trial area. This had increased to spread up to 3.5 m from a point source by 7 weeks.

Discussion

For some of the seed batches examined, *S. petroselini* was found to be non-viable. Maude (1996) demonstrated that viability of *S. apiicola* on celery seed can decline over time; loss in viability is more rapid under conditions of high temperature and high relative humidity. Decline in pathogen viability over time could have occurred for seed lots of parsley harvested in 2004 (e.g. seed lot A). However, seed age is less likely to account for loss in the viability of *S. petroselini* for seed lots harvested in 2005 and 2006, and subsequently stored under cool conditions.

The results demonstrate that neither the percentage of seeds with pycnidia nor the percentage of seeds showing spore release from pycnidia give a useful measure of pathogen viability or subsequent risk to a parsley crop. Finding a reliable measurement of the percentage seeds with viable infection is now further confounded by the result that spores of *S. petroselini* are not just restricted to parsley seeds with pycnidia but can also be found on seeds that are visibly free from pycnidia. In summary, a seed batch with pycnidia could pose little risk, while a seed lot that is apparently healthy could contain viable spores. These findings may impact on future seed testing methods for parsley Septoria.

Transmission of *S. petroselini* to seedlings was demonstrated only using the seed lot for which the pathogen was shown to be viable. Therefore, the transmission rate for *S. petroselini* from seed to seedling was estimated using a single seed lot. Despite subsequent use of a seed lot artificially infested with different doses of viable spores, transmission to seedlings did not occur, such that further verification of transmission rates was not possible. The lack of disease transmission following artificial inoculation is difficult to explain; based on the results of the first transmission experiment, and assuming the smallest value for the one-hit probability, even the lowest inoculum dose would be expected to give pathogen transmission. It is possible that spores on artificially infested seeds did not remain viable for long enough to achieve transmission from seed to seedling.

The disease spread experiment was useful in demonstrating the extent to which parsley Septoria can spread under environmental conditions that are high risk for disease development. It would be possible to fit models to the spread data and to transmission data obtained from one naturally infested seed lot. However, without further information on dose-response relationships, this approach would currently be of limited value.

Evaluation of fungicides for control of parsley Septoria

Introduction

Results on the efficacy of different fungicide products against parsley Septoria when applied at different timings in relation to infection and symptom development were presented in the Year 2 project report. In summary:

- In artificially inoculated pot experiments, the following fungicide products significantly reduced the incidence and severity of parsley Septoria caused by *S. petroselini*: Amistar (azoxystrobin), Signum (boscalid + pyraclostrobin), Folicur (tebuconazole) and Karamate Dry Flo Newtec (mancozeb). Mancozeb was the most effective fungicide, reducing mean disease incidence to 14% at 34 days after inoculation compared to 100% in the untreated control.
- Products were applied either 5 days before, 2 days before or 2 days after artificial inoculation. Overall, fungicides were most effective when applied 2 days before or 2 days after inoculation. Amistar was most effective when applied 2 days before inoculation, compared with other timings. Karamate Dry Flo Newtec was effective even when applied 5 days before inoculation.
- When fungicides were applied to lesions of Septoria leaf spot containing mature pycnidia, all fungicides tested except Switch (cyprodinil + fludioxonil) reduced spore germination, with Amistar and Signum being particularly effective.

The aim of this experiment was to evaluate fungicide programmes for the control of parsley Septoria on an 8-week crop. The most effective and approved products were selected from year 2 work, and evaluated as three-spray programmes commencing either before, or after an infection event.

Materials and Methods

The experiment was sited in a polytunnel at ADAS Arthur Rickwood, Cambs. The crop used was of a curly leaf parsley variety (Bravour) that had been planted using parsley seedlings from modules in November 2008. The crop was planted almost continuously along the row (3 cm spacing) with 20 cm between rows, giving an area of 21 rows and 7 m length. The crop was cut back in May 2009 and used for the disease spread experiment described previously. Once the spread experiment was complete, the crop was again cut back to stem bases (11 August 2009) and foliage removed from the polytunnel, before plots were marked out and paths between replicate blocks ‘burned out’ using glyphosate.

The experiment comprised a randomised complete block design with four replicate blocks. Each plot was four rows wide (20 cm spacing) and 1 m long (3 cm plant spacing).

The experimental area had been previously infected with *S. petroselini* during the spread experiment although foliage in the central rows (where infection had been most severe) had been ‘burned off’ with glyphosate to create pathways, and remaining foliage had been cut back to stem bases and removed. To augment residual inoculum in the trial area, artificial inoculum was prepared as follows for application on the same day: sporulating sub-cultures

of *S. petroselini* were prepared on V8 agar. The plates were scraped with a sterile spatula after pouring approx 5 ml sterile distilled water onto each plate. In addition, dried leaves of parsley with typical symptoms of Septoria leaf spot were immersed in distilled water and soaked for 1 h, agitating regularly. The resulting spore suspension from plate cultures and leaves was filtered through sterile muslin. The concentration was 3.25 x 10^5 spores/ml measured using a haemocytometer and microscopes. To test inoculum viability, 50 µl spore suspension was pipetted onto each of three plates of PDA+S and examined for percentage spore germination (100 spores per plate checked for germ tube development under x400 magnification) after incubation for 16 h at approximately 20^{o}C.

The plots of parsley were inoculated on 4 September 2009 (once re-growth had occurred) by spraying to run-off with the spore suspension, using a pump action sprayer (approx 0.5 L per replicate block). In order to provide conditions conducive for disease development (prolonged leaf wetness), the plots were covered with a polythene tent for 3 days. Once the polythene was removed the plots were misted twice daily using an overhead misting irrigation system for 10 min.

There were six fungicide programmes plus an untreated control as shown in Table 11. Three of the fungicide programmes commenced 4 days before artificial inoculation with *S. petroselini*. The remaining programmes commenced 4 days after inoculation. Fungicides were applied in 1000 L water/ha (100 ml/m^2) using an Oxford precision sprayer with single nozzle (plus guard to prevent spray drift) at 2 Bar pressure.

Table 11. Fungicide programmes applied to parsley, Cambs

	Spray 1 31.08.09	Inoculate 04.09.09	Spray 2 08.09.09	Spray 3 18.09.09	Spray 4 28.09.09
1	Untreated	Yes	Untreated	Untreated	Untreated
2	Karamate	Yes	Karamate	Karamate	-
3	Amistar	Yes	Amistar	Karamate	-
4	Signum	Yes	Signum	Karamate	-
5	-	Yes	Karamate	Karamate	Karamate
6	-	Yes	Amistar	Amistar	Karamate
7	-	Yes	Signum	Signum	Karamate

Details of products used in parsley spray trial

Product	Active ingredient	Product rate	Approval
Amistar	Azoxystrobin	1 L/ha	SOLA 1293/02
Signum	Boscalid + pyraclostrobin	1.5 kg/ha	SOLA 1984/04
Karamate Dry Flo Newtec	Mancozeb	3.9 kg/ha	SOLA 0028/10

Disease incidence and severity were assessed 4 days before inoculation, then at 14, 24, 31 and 38 days after inoculation. For each plot, five x 10 cm row lengths were assessed, using the middle two rows of each plot and avoiding the end 20 cm of each plot. For each row length, the presence or absence of Septoria was determined, and the percentage leaf area affected. At each assessment, plants were visually examined for spray deposits, symptoms of phytotoxicity or growth benefits in comparison with the untreated control. Data for disease severity (% leaf area affected by symptoms) and disease incidence (% row lengths affected) were analysed by ANOVA (using a logit transformation) and Generalised Linear Models respectively, in Genstat .

Results and discussion

The viability of spores of *S. petroselini* used for artificial inoculation was >98%.

At the first assessment (4 days before inoculation), no disease symptoms were present on any treatments. Symptoms of Septoria were first observed 14 d after artificial inoculation. Disease progress (as mean % incidence) is shown in Figure 7.

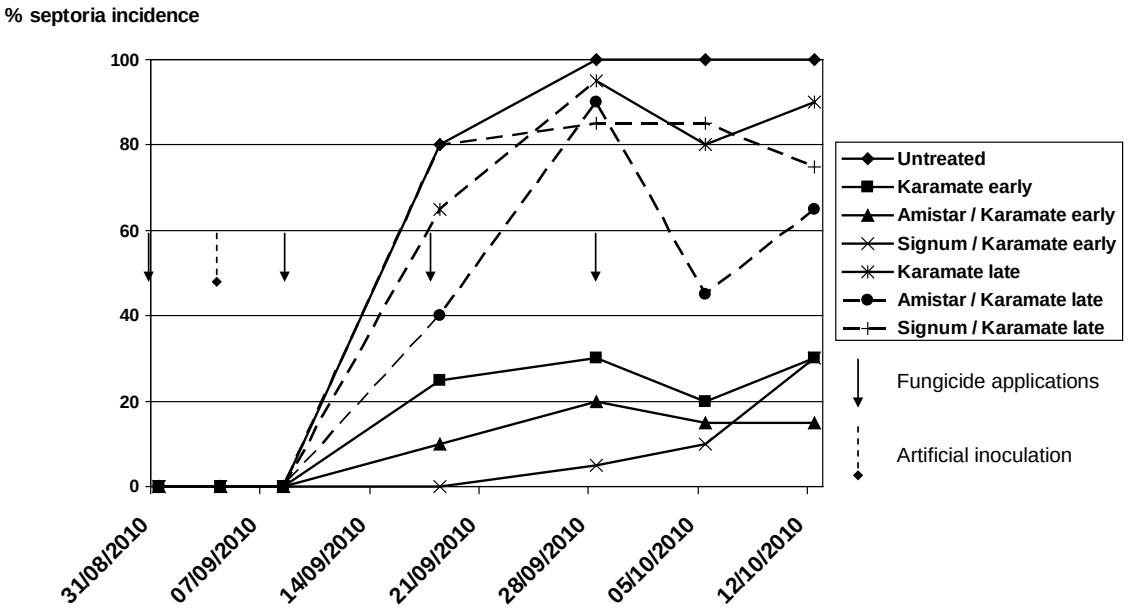


Figure 7. Development of parsley septoria following fungicide applications and artificial inoculation with *Septoria petroselini*, Cambs 2009.

There was a significant effect of fungicide programme on disease incidence at all assessment dates (see Appendix 4). At 14 d after inoculation, treatments 3 and 4 had reduced Septoria incidence compared with the untreated control (Table 12). At the time of assessment, these treatments had each received two applications of strobilurin fungicides, commencing before inoculation. Treatment 4, with two sprays of Signum, had no disease symptoms compared with 80% incidence in the untreated control. At the end of the experiment (38 d after inoculation), all of the spray programmes that had commenced before artificial inoculation significantly reduced disease incidence compared with the untreated control. Treatments 6 and 7 (two strobilurins, then mancozeb) commencing after inoculation also reduced disease incidence compared with the control.

Disease severity remained low for the duration of the experiment, not exceeding a mean severity of 7% in the untreated control plots. At 14 days after inoculation, treatments 3, 4 and 5 that had commenced before inoculation had lower Septoria severity than the untreated control (Table 13; Appendix 4). At the end of the experiment (38 d after inoculation), all of the spray programmes significantly reduced disease severity compared with the untreated control. In addition, treatments 3, 4, 5 that started before inoculation were more effective in reducing severity than treatments 6, 7 and 8 that commenced later.

Table 12. Effect of fungicide programmes on the incidence of parsley Septoria, Cambs 2009.

	Fungicide programme	Commencing before or after inoculation	Mean % incidence Septoria* (s.e. in parentheses)	
			18.09.09 14 d after inoculation	12.10.09 38 d after inoculation
1	Untreated	-	80 (15.1)	100 (-)
2	Karamate x 3	Before	25 (16.2)	30 (11.6)
3	Amistar x 2, Karamate	Before	10 (11.3)	15 (9.1)
4	Signum x 2, Karamate	Before	0 (-)	30 (11.6)
5	Karamate x 3	After	65 (17.9)	90 (7.6)
6	Amistar x 2, Karamate	After	40 (18.3)	65 (12.0)
7	Signum x 2, Karamate	After	80 (15.1)	75 (10.9)

*Mean of four replicate plots, with five assessments per plot

Table 13. Effect of fungicide programmes on the severity of parsley Septoria, Cambs 2009

	Fungicide programme	Commencing before or after inoculation	Mean % Septoria severity*	
			18.09.09 14 d after inoculation	12.10.09 38 d after inoculation
1	Untreated	-	0.27	6.88
2	Karamate x 3	Before	0.02	0.07
3	Amistar x 2, Karamate	Before	0.01	0.05
4	Signum x 2, Karamate	Before	0.00	0.07
5	Karamate x 3	After	0.14	0.84
6	Amistar x 2, Karamate	After	0.23	0.34
7	Signum x 2, Karamate	After	0.22	0.44

There were no phytotoxic effects or growth benefits due to the fungicide programmes applied. However, there were some spray residues left on leaves following application of Karamate Dry Flo Newtec, such that use of this chemical as the last application in a spray programme would need to be avoided. The harvest interval for the three products used in the experiment is 14 days.

The results confirm previous experiments from project year 2, indicating that Amistar, Signum and Karamate Dry Flo Newtec are very effective fungicide products for the control of parsley Septoria. In this experiment, three applications of these products over an 8-week period were sufficient to control Septoria even under very high inoculum pressure (when applied as protectants). Sprays were applied at intervals of 8-10 days; under dry conditions, there may be scope for reducing the number and/or frequency of spray applications.

Project summary and conclusions

Coriander seed testing, transmission, spread and seed health standards

- A significant proportion of coriander seed lots tested were infested with *Pseudomonas syringae* pv. *coriandricola* (*Psc*)
- Estimates were obtained for seed-to-seedling transmission probabilities for coriander/bacterial blight.
- Bacterial blight spread from a single point source (equivalent to transmission by 1 in 15,000 seeds) to almost 30% of the crop by 57 days after sowing.
- Parameter estimates were obtained for a model describing the spread of bacterial blight in the field.
- Using these parameter estimates, transmission and spread models were combined with a seed test model to examine the impact of different seed health scenarios on disease in the field and estimate the risk of detection failure for different seed health assay designs.
- Based on the above modelling, growers should ideally only use coriander seed which has been tested for *Psc* to a tolerance standard of <0.03% (with 95% probability) with a detection limit of 900 CFU per 3000 seeds. This requires testing of at least 3 sub-samples of 3,000 seeds (9,000 total).
- The above standard is for individual outdoor fresh leaf crops, a more stringent standard may be appropriate for seed/spice crops and/or glasshouse pot-grown crops.
- Seed health test results for *Pseudomonas syringae* pv. *coriandricola* obtained by some laboratories may be of doubtful validity.
- It is vital that adequate primary samples are drawn from throughout the seed bulk when collecting samples for testing/analysis – follow ISTA sampling recommendations.

Coriander seed treatments

- Initial seed treatments and evaluations were conducted with hot water, thyme oil, chlorine dioxide and two BCAs.
- Hot water treatment at 53 or 52°C for 30 min reduced seed infestation levels to acceptable levels (i.e. below the recommended tolerance standard) in 5 out of 6 naturally infested seed lots.
- Thyme oil also gives a useful reduction, but is phytotoxic at higher concentrations and less effective at lower concentrations.
- Chlorine dioxide at the concentrations used (100 and 500 ppm) appeared to have no effect.
- The two BCAs (Subtilex and Serenade Max) were evaluated in glasshouse transmission experiments using both inoculated and naturally infected seed lots.

- Both BCAs appear to give a reduction in transmission of the pathogen.

Parsley seed testing, transmission and spread

- Results from seed testing demonstrated that neither the percentage of seeds with pycnidia nor the percentage of seeds showing spore release from pycnidia give a useful measure of pathogen viability or subsequent risk to a parsley crop.
- A seed lot with pycnidia could pose little risk to crop health, while a seed lot that is apparently healthy (i.e. without visible pycnidia) could contain viable spores.
- These findings show that development of a standard seed testing method for parsley Septoria must take account of the viability of spores on seed lots, rather than relying on presence of pycnidia and spores alone.
- Transmission of *S. petroselini* from seed to seedlings was demonstrated only using one seed lot for which the pathogen was previously shown to be viable. Estimates of the one-hit probability of transmission (probability of transmission of one spore on one seed) varied, with values of 9.0×10^{-5}, 8.7×10^{-5} or 6.2×10^{-5}, depending on whether primary infection foci were considered at the seedling, cell or cluster level, respectively.
- To further verify this rate and to determine dose response, transmission studies were done with parsley seed artificially infested with varying doses of *S. petroselini* spores, however, no symptoms of Septoria developed on seedlings from these seed lots.
- Under environmental conditions conducive for disease development, Septoria spread 3.5 m within a parsley crop from a single point of infection in 7 weeks.

Parsley seed treatments

- Alternative seed treatments for parsley Septoria could not be evaluated in this project due to lack of seed lots with viable *S. petroselini.*
- Parsley seed can currently be treated for Septoria using a warm water thiram soak.

Foliar fungicides for parsley Septoria

- In artificially inoculated pot experiments, the following fungicides were effective against parsley Septoria caused by *S. petroselini*: Amistar (azoxystrobin), Signum (boscalid + pyraclostrobin), Folicur (tebuconazole) and Karamate Dry Flo Newtec (mancozeb). Mancozeb was the most effective of these products.
- Amistar was most effective when applied 2 days before inoculation, compared with other timings. Karamate Dry Flo Newtec was effective even when applied 5 days before inoculation.
- When fungicides were applied to lesions of Septoria leaf spot containing mature pycnidia, all fungicides tested except Switch (cyprodinil + fludioxonil) reduced spore germination, with Amistar and Signum being particularly effective.

- Under high inoculum pressure, programmes alternating either Amistar or Signum with Karamate Dry Flo Newtec (all with SOLAs for outdoor parsley), were highly effective in reducing Septoria, particularly when programmes commenced before infection had occurred.

Potential forecasting approaches for parsley Septoria

- Available information on environmental conditions conducive for the development of parsley Septoria, and possible forecasting approaches was summarised in project year 1.
- There are no models that have been developed specifically for predicting the development of Septoria on parsley. Of models reviewed, the Tom-Cast system has the advantage that it has been validated for use in a range of crop/disease situations, and has been shown to enable reduced spray numbers in certain seasons while still maintaining marketable quality.
- The disadvantage of the Tom-Cast model is that it relies on previous weather conditions rather than forecast conditions, such that fungicides with strong curative activity (not available for parsley) would be required.
- On a short duration crop such as parsley, for which quality standards are high, growers would be advised to apply protectant sprays of fungicides effective for parsley Septoria control when high risk conditions are forecast (particularly prolonged wet periods). Spray intervals can be extended or numbers of applications reduced during dry conditions because Septoria risk is lower.

Approval status of treatments/products used

Coriander seed treatments

Hot water treatment does not require approval.

Serenade MAX (a powdered formulation of a *Bacillus subtilis* strain) does not have approval as a plant protection product in the UK; it is registered as a fungicide in the USA. An alternative formulation Serenade ASO has recently received approval as a plant protection product in the UK, with a SOLA allowing application to a broad range of crops, but it is not approved as a seed treatment.

Subtilex (a *Bacillus subtilis* strain) does not have approval as a plant protection product in the UK; it is registered as a biological fungicide in the USA.

Chlorine dioxide is a biocide and does not have approval as plant protection product in the UK.

Thyme oil (white, FCC) does not have approval as a plant protection product in the UK.

Fungicides for parsley Septoria

Parsley seed can currently be treated for Septoria using a warm water thiram soak. None of the fungicides approved for foliar treatment of parsley leaf spot are approved as seed treatment formulations.

The approval status of fungicides that were effective in this project for control of Septoria on outdoor parsley is summarised below:

Product	Active ingredient	Approval
Amistar	Azoxystrobin	SOLA 1293/02
Signum	Boscalid + pyraclostrobin	SOLA 1984/04
Karamate Dry Flo Newtec	Mancozeb	SOLA 0028/10

Recommendations for Further work

- Parameters for the coriander seed to seedling transmission model are based on limited data – additional experiments would be useful to improve the robustness of the estimates and the recommended seed health standard.
- Serenade has approval as a foliar treatment, growers may wish to consider funding a small trial to determine whether there is any benefit from post-emergence treatment of coriander for the control of coriander bacterial blight.
- Seed health standards for parsley Septoria could not be fully developed because of limited pathogen viability on seed lots obtained and limited transmission from seed to seedling. Additional experiments would be useful to determine the dose-response relationships and to investigate the relative contribution of seed with and without pycnidia to transmission.

Technology transfer (year 3)

- A Factsheet on coriander bacterial blight is ready for publication and a factsheet on parsley Septoria is in preparation
- Presentations on the coriander bacterial blight and parsley Septoria were given at the BHTA Technical Meeting on 3 March 2010.
- An article has been submitted for publication in the September edition of HDC News.
- A poster on some aspects of the work on coriander bacterial blight will be presented at the 8th International Conference on *Pseudomonas syringae* pathovars

Acknowledgements

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Appendix 1. Statistical analyses – coriander seed health standards

Genstat output for estimation of one-hit probability

Regression analysis
==========

Response variate: Pos
Binomial totals: 1
Distribution: Binomial
Link function: Complementary log-log
Offset variate: LogN
Fitted terms: Constant, Logd

Estimates of parameters

Parameter	estimate	s.e.	t(64)
Constant	-8.72	1.25	-6.99
Logd	0.2818	0.0889	3.17

* MESSAGE: s.e.s are based on the residual deviance.

Accumulated analysis of deviance

Change	d.f.	mean deviance	deviance	approx ratio	F pr.
+ Logd	1	6.3873	6.3873	13.00	<.001
Residual	64	31.4329	0.4911		
Total	65	37.8202	0.5818		

Genstat output for estimation of disease spread parameters

Summary of analysis

Source	d.f.	deviance	mean deviance	approx ratio	chi pr
Regression	2	501.2	250.5935	250.59	<.001
Residual	1801	354.5	0.1968		
Total	1803	855.7	0.4746		

R-squared statistic (based on deviance) 0.586

* MESSAGE: deviance ratios are based on dispersion parameter with value 1.

Estimates of parameters

Parameter	estimate	s.e.	t(*)	antilog of estimate
Constant	-13.03	1.24	-10.50	0.000002202
logdist	-3.393	0.320	-10.59	0.03362
day	0.2724	0.0266	10.22	1.313

Appendix 2. Statistical analyses – coriander seed treatment

Estimation of bacterial numbers for physical/chemical treatments

Regression analysis
==========

Response variate: Count
Distribution: Poisson
Link function: Log
Weight variate: N_seed
Offset variate: lnOff
Fitted terms: Constant, Samp

Estimates of parameters

Parameter	estimate	s.e.	t(44)	antilog of estimate
Constant	-0.99	6.82	-0.15	0.3713
Samp 50-30	1.69	6.96	0.24	5.420
Samp 55-15	-6.1	39.1	-0.16	0.002211
Samp 55-30	-6.1	39.1	-0.16	0.002213
Samp Cl100	10.28	6.83	1.50	29048.
Samp Cl500	8.43	6.89	1.22	4580.
Samp S1072	10.65	6.82	1.56	42268.
Samp Thyme	4.42	7.13	0.62	83.45

* MESSAGE: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Samp	50-15

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	approx ratio	F pr.
+ Samp	7	76135309.	10876473.	88.67	<.001
Residual	44	5396873.	122656.		
Total	51	81532183.	1598670.		

Predictions from regression model

These predictions are estimated mean values, formed on the scale of the linear predictor.

The predictions have been formed only for those combinations of factor levels that are present in the data.

The predictions are based on a supplied value for the offset variate:
lnOff 0.

The standard errors are appropriate for interpretation of the predictions as summaries of the data rather than as forecasts of new observations.

Response variate: Count

Samp	p	s
50-15	-0.991	6.816
50-30	0.699	1.428
55-15	-7.105	38.510
55-30	-7.104	38.528
Cl100	9.286	0.462
Cl500	7.439	0.980
S1072	9.661	0.139
Thyme	3.434	2.092

* MESSAGE: s.e's, variances and lsd's are approximate, since the model is not linear.
* MESSAGE: s.e's are based on the residual deviance.

Transformed to logs to base 10:

Samp	p	s
50-15	-0.430	2.960
50-30	0.304	0.620
55-15	-3.086	16.725
55-30	-3.085	16.733
Cl100	4.033	0.201
Cl500	3.231	0.426
S1072	4.196	0.060
Thyme	1.491	0.908

Estimation of bacterial numbers for biological treatments

Regression analysis
==========

Response variate: Count
Distribution: Poisson
Link function: Log
Weight variate: N_seed
Offset variate: lnOff
Fitted terms: Constant + Lot + Treat + Lot.Treat

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	approx ratio	F pr.
+ Lot	1	11519740.	11519740.	1644.54	<.001
+ Treat	2	126650.	63325.	9.04	<.001
+ Lot.Treat	2	1061.	530.	0.08	0.927
Residual	90	630435.	7005.		
Total	95	12277886.	129241.		

Predictions from regression model

These predictions are estimated mean values, formed on the scale of the linear predictor.

The predictions have been formed only for those combinations of factor levels that are present in the data.

The predictions are based on a supplied value for the offset variate:
lnOff 0.

The standard errors are appropriate for interpretation of the predictions as summaries of the data rather than as forecasts of new observations.

Response variate: Count

Treat	Serenade		Subtilex	
	p	s	p	s
Lot				
S1072	-4.342	52.722	2.351	2.647
S1081	12.991	0.151	13.340	0.137

Treat	Untreated	
	p	s
Lot		
S1072	2.957	2.041
S1081	13.791	0.120

* MESSAGE: s.e's, variances and lsd's are approximate, since the model is not linear.

* MESSAGE: s.e's are based on the residual deviance.

Converted to logs to base 10:

Treat	Serenade		Subtilex	
	p	s	p	s
Lot				
S1072	-1.886	22.897	1.021	1.149
S1081	5.642	0.066	5.793	0.060

Treat	Untreated	
	p	s
Lot		
S1072	1.284	0.886
S1081	5.989	0.052

Appendix 3. Seed health standards for parsley Septoria

Characterisation of parsley seed lots to determine infection levels of Septoria petroselini

Seed lot	Rep	% seed with visible pycnidia			% seed with spore release at 0 h			% seed with spore release at 24 h			% seed with spore germination at 24 h		
			Mean	S.d.		Mean	S.d.		Mean	S.d.		Mean	S.d.
A	1	43			5			22			0		
	2	44			1			5			0		
	3	36			0			9			0		
	4	38	40.3	3.86	0	1.5	2.38	3	9.8	8.54	0	0.0	0.00
B	1	49			17			40			0		
	2	36			17			41			0		
	3	26			11			17			0		
	4	26	34.3	10.90	9	13.5	4.12	26	31.0	11.58	0	0.0	0.00
C	1	1			1			1			0		
	2	0			0			0			0		
	3	1			0			0			0		
	4	0	0.5	0.58	0	0.3	0.50	0	0.3	0.50	0	0.0	0.00
D	1	14			7			10			1		
	2	4			0			5			0		
	3	4			0			2			0		
	4	3	6.3	5.19	0	1.8	3.50	3	5.0	3.56	0	0.3	0.50
E	1	0			0			0			0		
	2	0			0			0			0		
	3	5			0			0			0		
	4	3	2.0	2.45	0	0.0	0.00	0	0.0	0.00	0	0.0	0.00
F	1	0			0			0			0		
	2	0			0			0			0		
	3	0			0			0			0		
	4	2	0.5	1.00	0	0.0	0.00	0	0.0	0.00	0	0.0	0.00
G	1	11			4			6			6		
	2	11			0			7			0		
	3	10			3			13			1		
	4	12	11.0	0.82	1	2.0	1.83	5	7.8	3.59	1	2.0	2.71

Incidence and distribution of S. petroselini in a module tray of parsley seedlings from seed batch code G, 45 days after exposure to conditions favourable to disease development

Number of diseased seedlings in cell

0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	2	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Total diseased : 25 (* seed with pycnidia attached)

Number of seedlings per cell

0	3	4	2	2	2	1	1	1	5	5	1	3	3	1	1	2	3	2	3	1
0	3	2	2	4	6	2	4	2	1	2	0	3	5	2	2	1	3	4	2	1
0	5	4	1	0	2	1	3	3	1	4	2	4	4	5	4	5	2	1	2	2
1	4	3	1	3	3	1	4	0	2	2	0	1	2	2	2	2	3	3	2	2
2	2	1	1	3	3	3	4	2	2	2	3	4	3	3	2	3	3	3	3	1
2	1	0	2	1	4	2	0	3	3	5	5	4	3	3	3	3	3	4	3	0
1	2	2	1	4	3	0	1	1	3	3	3	2	3	4	5	1	2	5	3	1
1	1	3	2	3	2	2	2	2	2	2	3	3	3	4	4	3	2	1	3	0
3	4	1	3	3	3	3	2	4	4	5	4	3	0	4	4	3	0	5	3	1
2	2	3	3	2	4	2	3	1	2	1	1	3	3	2	2	3	2	3	3	2
2	3	1	3	2	4	3	4	4	2	2	5	4	3	2	2	2	3	1	3	3
3	4	1	2	3	3	4	3	2	4	3	0	4	3	3	2	3	2	3	1	0
4	2	4	3	3	0	2	0	2	2	2	2	3	1	4	4	2	2	2	1	2
3	1	1	1	2	2	2	4	2	1	4	4	2	3	3	3	3	3	1	3	1

Total no. seedlings : 718 **% diseased : 3.48**

Appendix 4. Statistical analyses – fungicide programmes for parsley Septoria

Regression analysis (disease incidence)

Response variate: Incidence_Ass1 (14 days after inoculation)
Binomial totals: 5
Distribution: Binomial
Link function: Logit
Fitted terms: Constant, Block, Treat

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Block	3	1.628	0.543	0.19	0.903
+ Treat	6	63.904	10.651	3.70	0.014
Residual	18	51.825	2.879		
Total	27	117.357	4.347		

Predictions from regression model

Response variate: Incidence_Ass1

Treat	Prediction	s.e.
1	0.8000	0.1505
2	0.2500	0.1621
3	0.1000	0.1130
4	0.0000	0.0012
5	0.6500	0.1786
6	0.4000	0.1829
7	0.8000	0.1505

Regression analysis

Response variate: Incidence_Ass2 (24 days after inoculation)
Binomial totals: 5
Distribution: Binomial
Link function: Logit
Fitted terms: Constant, Block, Treat

Accumulated analysis of deviance

Change	d.f.	mean deviance	approx deviance	ratio	F pr.
+ Block	3	0.3285	0.1095	0.14	0.935
+ Treat	6	97.8411	16.3069	20.67	<.001
Residual	18	14.1983	0.7888		
Total	27	112.3679	4.1618		

Predictions from regression model

Response variate: Incidence_Ass2

Treat	Prediction	s.e.
1	1.0000	0.00035
2	0.3000	0.09046
3	0.2000	0.07909
4	0.0500	0.04323
5	0.9500	0.04321
6	0.9000	0.05940

7	0.8500	0.07062

Regression analysis

Response variate:	Incidence_Ass3 (31 days after inoculation)
Binomial totals:	5
Distribution:	Binomial
Link function:	Logit
Fitted terms:	Constant, Block, Treat

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Block	3	4.954	1.651	1.31	0.303
+ Treat	6	84.365	14.061	11.12	<.001
Residual	18	22.769	1.265		
Total	27	112.088	4.151		

Predictions from regression model

Response variate: Incidence_Ass3

Treat	Prediction	s.e.
1	1.0000	0.00075
2	0.2000	0.09530
3	0.1500	0.08590
4	0.1000	0.07307
5	0.8000	0.09732
6	0.4500	0.11699
7	0.8500	0.08753

Regression analysis

Response variate:	Incidence_Ass4 (38 days after inoculation)
Binomial totals:	5
Distribution:	Binomial
Link function:	Logit
Fitted terms:	Constant, Block, Treat

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Block	3	1.717	0.572	0.43	0.731
+ Treat	6	64.564	10.761	8.16	<.001
Residual	18	23.726	1.318		
Total	27	90.006	3.334		

Predictions from regression model

Response variate: Incidence_Ass4

Treat	Prediction	s.e.
1	1.0000	0.00075
2	0.3000	0.11621
3	0.1500	0.09106
4	0.3000	0.11621
5	0.9000	0.07640
6	0.6500	0.12035
7	0.7500	0.10948

Analysis of variance (disease severity)

Variate: logitSevAss1 (14 days after inoculation)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	3	0.34096	0.11365	1.84	0.176
Treat	6	0.84599	0.14100	2.29	0.081
Residual	18	1.11067	0.06170		
Total	27	2.29762			

Message: the following units have large residuals.

units 11	-0.407	s.e.	0.199
units 13	0.606	s.e.	0.199

Tables of means

Variate: logitSevAss1

Grand mean -5.079

Block	1	2	3	4
	-5.109	-4.891	-5.154	-5.162

Treat	1	2	3	4	5	6	7
	-4.858	-5.245	-5.274	-5.293	-5.038	-4.913	-4.932

Standard errors of differences of means

Table	Block	Treat
rep.	7	4
d.f.	18	18
s.e.d.	0.1328	0.1756

Least significant differences of means (5% level)

Table	Block	Treat
rep.	7	4
d.f.	18	18
l.s.d.	0.2790	0.3690

Treat	**BackMn1**
1	0.2707
2	0.0244
3	0.0097
4	0.0000
5	0.1444
6	0.2300
7	0.2160

Analysis of variance

Variate: logitSevAss2 (24 days after inoculation)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	3	1.2146	0.4049	2.20	0.123
Treat	6	22.5809	3.7635	20.45	<.001
Residual	18	3.3127	0.1840		
Total	27	27.1082			

Message: the following units have large residuals.

units 27	1.001	s.e.	0.344

Tables of means

Variate: logitSevAss2

Grand mean -4.468

Block	1	2	3	4
	-4.446	-4.640	-4.651	-4.136

Treat	1	2	3	4	5	6	7
	-2.545	-5.235	-5.254	-5.247	-4.110	-4.411	-4.476

Standard errors of differences of means

Table	Block	Treat
rep.	7	4
d.f.	18	18
s.e.d.	0.2293	0.3033

Least significant differences of means (5% level)

Table	Block	Treat
rep.	7	4
d.f.	18	18
l.s.d.	0.4818	0.6373

Treat	**BackMn2**
1	6.779
2	0.030
3	0.020
4	0.023
5	1.115
6	0.700
7	0.625

Analysis of variance

Variate: logitSevAss3 (31 days after inoculation)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	3	2.1066	0.7022	4.33	0.018
Treat	6	23.6007	3.9335	24.24	<.001
Residual	18	2.9209	0.1623		
Total	27	28.6283			

Message: the following units have large residuals.

units 11	-0.672	s.e.	0.323

Tables of means

Variate: logitSevAss3

Grand mean -4.465

Block	1	2	3	4
	-4.472	-4.815	-4.526	-4.047

Treat	1	2	3	4	5	6	7
	-2.447	-5.184	-5.228	-5.238	-4.213	-4.658	-4.289

Standard errors of differences of means

Table	Block	Treat
rep.	7	4
d.f.	18	18
s.e.d.	0.2153	0.2848

Least significant differences of means (5% level)

Table	Block	Treat
rep.	7	4
d.f.	18	18
l.s.d.	0.4524	0.5984

Treat	**BackMn3**
1	7.468
2	0.058
3	0.034
4	0.028
5	0.959
6	0.440
7	0.853

Analysis of variance

Variate: logitSevAss4 (38 days after inoculation)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	3	0.47333	0.15778	2.25	0.118
Treat	6	21.50543	3.58424	51.01	<.001
Residual	18	1.26475	0.07026		
Total	27	23.24351			

Tables of means

Variate: logitSevAss4

Grand mean -4.540

Block	1	2	3	4
	-4.473	-4.668	-4.661	-4.360

Treat	1	2	3	4	5	6	7
	-2.529	-5.167	-5.193	-5.164	-4.302	-4.767	-4.660

Standard errors of differences of means

Table	Block	Treat
rep.	7	4
d.f.	18	18
s.e.d.	0.1417	0.1874

Least significant differences of means (5% level)

Table	Block	Treat
rep.	7	4
d.f.	18	18
l.s.d.	0.2977	0.3938

	BackMn4
Treat	
1	6.884
2	0.067
3	0.052
4	0.069
5	0.837
6	0.344
7	0.438