Project Title:	Outdoor herbs: Integrated management of parsley Septoria and coriander bacterial blight
Project number:	FV 318
Project leader:	Dr Kim Green, ADAS
Report:	Annual report, April 2008
Previous report	None
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Date project commenced:	01 May 2007
Date completion due:	30 April 2010
Keywords:	Herbs, disease, fungi, bacteria, parsley, *Septoria petroselini*, coriander, bacterial leaf spot, seed, fungicides, bacteria, *Pseudomonas syringae* pv. *coriandricola*, seed testing, seed health, control

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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Research Scientist
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GROWER SUMMARY

Headline

- A significant proportion of coriander seed lots tested were found to be infested with the coriander bacterial blight pathogen (*Psc*).
- Tests with parsley seed lots infested with *Septoria petroselini* demonstrated that neither the percentage of seeds with spore cases nor the percentage of seeds showing spore release provides a reliable measure of pathogen viability or subsequent disease risk to the crop.
- First estimates have been obtained for seed to seedling transmission probabilities for both coriander/bacterial blight and parsley/Septoria.

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*).

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 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 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.
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 factsheet on integrated strategies for management of parsley Septoria and coriander leaf blight.

This report contains the results of work done during the first year of the project.

Summary of results and conclusions

Coriander seed testing

Nine coriander seed lots were tested for the presence of the bacterial blight pathogen (*Psc*) using a method developed by Plant Health Solutions. Six out of the nine coriander seed lots examined were found to be infested with *Psc*. Infestation levels together with upper and lower confidence limits, are shown in Table 1, and ranged from 0.4 to 5% in infested seed lots. It is important to note that in the lots where *Psc* was not detected and in common with all seed health tests, it is not possible to be completely certain that they are healthy. This is due to the effects of sampling and the detection limits inherent in the test method. Therefore, for lots where *Psc* was not detected only an upper 95% confidence limit is provided. This implies that we can be 95% confident that the true infestation level is below this limit.

The seed test results indicate that a significant proportion of coriander seed lots may be infested with *Psc*. This would go some way to explaining the prevalence of this disease and perhaps also gives some optimism that improving seed health through a programme of effective testing to achieve a defined tolerance standard will go someway

to reducing the problems caused by coriander bacterial blight. Work done in this and subsequent years will attempt to define such a tolerance standard.

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

Sample No.	% of seeds infested		
	Mean	95% confidence limits	
		Lower	Upper
S1040	<0.03	0.00	0.03
S1041	<0.02	0.00	0.02
S1042	0.7	0.12	4.6
S1043	3.9	0.37	22
S1044	5.0	0.40	33
S1045	4.4	0.38	26
S1046	<0.015	0.00	0.015
S1047	0.7	0.12	4.6
S1060	>0.009	0.009	100

Some of the seed lots examined had apparently already been tested for bacterial blight but had been reported as negative. This highlights the importance of ensuring that seed health testing labs have the appropriate, experience, expertise and test methods for the pathogen in question. Growers should not assume that a particular laboratory has the expertise or methodology to perform a particular test and should always query the detection limits and test sensitivity of the method.

Coriander 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 we used a 'one-hit' theoretical model for infection, which 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 coriander transmission experiment used both naturally infested and artificially inoculated seed to look at dose/response relationship (i.e. the relationship between the numbers of bacteria on the seed and the proportion of plants emerging which are contaminated). Seed was inoculated with a range of doses of bacteria (from 2×10^1 to 7×10^4 bacteria or CFU per seed) in two different ways (to simulate surface and more deep-seated infestation). The seed was then sown in module trays and grown

on capillary matting to avoid water-splash and prevent plant to plant spread of the pathogen. Emerged plants were then assessed for the presence of the pathogen.

Transmission occurred at a lower frequency than expected and was only detected at the highest inoculum levels. As the two doses where transmission was detected were close together, it was not possible to fit a meaningful model to the data. The one-hit probability of transmission was therefore calculated separately for each of these treatments to provide a mean estimate of the one-hit probability of transmission of 3.6 x 10^{-7}. This cannot be considered to be a robust estimate, as the form of the dose/response relationship (i.e. the model) means that the data is relatively uninformative when all units are negative (i.e. when transmission is not detected). An additional transmission experiment is now underway with a higher dose than in the previous experiment.

Parsley seed testing

The objective was to determine for individual parsley seed lots:

- Percentage incidence of seeds with pycnidia (spore cases) of *Septoria petroselini*
- Percentage incidence of seeds that gave conidial (spore) release from pycnidia and subsequent germination
- Mean numbers of conidia per seed, using seed with and without pycnidia
- The frequency of transmission of *S. petroselini* from parsley seed to seedlings

Seven separate seed lots were used that were supplied by two commercial seed houses and reported to be infected with *S. petroselini.*

When seeds were examined microscopically, pycnidia (spore cases) of *S. petroselini* were visible on seed from all seven batches tested, with one batch containing 40% of seeds with pycnidia (Table 2). Spore release from pycnidia was observed for five of the seven seed lots (there was no spore release from lots E and F). However, subsequent germination of released spores (indicating pathogen viability) was only observed for two out of the seven lots (lots D and G).

Table 2. 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

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. 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.

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 no risk, while a seed lot that is apparently healthy (i.e. without visible pycnidia) could contain viable spores. These findings may impact on future seed testing methods for parsley Septoria.

Parsley seed transmission

As for coriander seed, 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.

The frequency of pathogen transmission was studied using seven seed lots supplied by two commercial seed houses and reported to be infected with *S. petroselini*. For each seed lot, seedlings in module trays were exposed to conditions conducive for Septoria in controlled environment cabinets and were subsequently monitored for lesion development.

Transmission of *S. petroselini* to seedlings was demonstrated only using the seed lot for which the pathogen was shown to be viable from seed testing results (lot G). 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. The transmission rate for *S. petroselini* from seed to seedling was estimated using a single seed lot. To further verify this rate, and to investigate further the relative contributions of seeds with and without pycnidia, seed lots with different mean doses of viable spores would be required.

Alternative seed treatment methods

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

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

Broad spectrum disinfectants/biocides are often considered as potential seed treatments for control of seedborne 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 treatments should not be investigated, but it should be made clear that pesticide approval would be required before their legal use could be permitted.

Several potential seed treatments have been identified and these will be examined in Year 2 of the project. These will include: hot water, thyme oil, at least one biological (e.g. Serenade™) and one conventional disinfectant (chlorine dioxide).

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. Forecasting models, based on temperature and leaf wetness duration, are available. These could potentially be validated or used as a basis for a simple decision-tree as an aid to spray timing for management of parsley Septoria. 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 developed across a wide temperature range.
- Under optimum conditions, symptoms of Septoria developed 9 days after inoculation.

There are no models that have been developed specifically for predicting the development of Septoria on parsley. However, there are three models that have been developed or adapted for the prediction of the closely related disease, Septoria late blight on celery (*S. apiicola*) and one model that has been developed for early blight of celery (*Cercospora apii*).

Of the 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 system utilises the duration of leaf wetness and average temperature during the wetness period to calculate a daily disease severity value (DSV). A fungicide is applied when the cumulative DSV reaches a predetermined threshold. The model has been validated for celery Septoria in Michigan state and Florida USA, and has also been introduced to celery growers in Victoria, Australia. Data can be downloaded from a temperature/leaf wetness sensor in the canopy and run through the Tom-Cast programme to provide a spray decision. It requires relatively inexpensive equipment, provides a straightforward output and has been implemented by growers. Use of Tom-cast for scheduling sprays for control of parsley Septoria will be trialled in project year 3.

Evaluation of fungicides for parsley Septoria

A replicated, inoculated pot experiment using flat leaf parsley was carried out from July 2007 to determine the relative protectant and curative activity of approved and novel fungicides applied at specified intervals before and after an infection event, for the control of parsley leaf spot. The experiment was repeated in October 2007, using curly leaf parsley.

Fungicide treatments were applied to parsley plants at four timings: 3 days before, 1 day before, 1 day after, or 3 days after artificial inoculation with *Septoria petroselini*. Products applied are shown in Table 3. It should be noted that Amistar Top does not have approval for use on outdoor herbs.

There were no phytotoxic effects or growth benefits following application of any of the fungicides to parsley in either of the experiments. The efficacy of the products against parsley Septoria could not be assessed because there was no development of Septoria on any of the parsley plants, despite use of viable inoculum. The experiment will be repeated in 2008, using modified conditions to increase the chance of disease development within experimental plots. Possible modifications include use of a higher concentration of spores and a longer period of leaf wetness following inoculation.

Table 3. Fungicide treatments evaluated against parsley Septoria

	Product	Active ingredient	Product rate
1	Untreated control	-	-
2	Amistar	Azoxystrobin	1 L/ha
3	Signum	Boscalid + pyraclostrobin	1.5 kg/ha
4	Scotts Octave	Prochloraz	0.2 kg/ha
5	Karamate Dry Flo Newtec	Mancozeb	3.9 kg/ha
6	Folicur	Tebuconazole	0.75 L/ha
7	Headland Inorganic Liquid Copper	Copper oxychloride	4.0 L/ha
8	Switch	Cyprodinil + fludioxonil	0.8 kg/ha
9	Amistar Top	Azoxystrobin + difenoconazole	1 L/ha

Notes:
Amistar – SOLA 1293/02
Signum – SOLA 1984/04
Octave – SOLA 0650/01
Karamate Dry Flo Newtec – SOLA 1978/06
Folicur – SOLA 1873/03
Headland Inorganic Liquid Copper – SOLA 1057/05
Switch – SOLA 2079/07
Amistar Top – Administrative Experimental Approval (use rate from SOLA 1476/06 on parsley root)

Financial benefits

None to date.

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 tolerance levels agreed with the supplier. Plant Health Solutions can provide such a testing service (see: www.seedtesting.co.uk).
- Be aware that seed testing results for parsley that quote percentage seeds infected with Septoria, may not provide a reliable measure of pathogen viability or disease risk to the crop.
- Parsley seed can be treated with Agrichem Flowable Thiram (thiram warm water soak) for the control of Septoria (follow label instructions).
- Broad spectrum disinfectants/biocides are not permitted for use as seed treatments for coriander or parsley.

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. 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.
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 factsheet on integrated strategies for management of parsley Septoria and coriander leaf blight

This report contains the results of work done during the first year of the project.

Developing appropriate seed health standards for coriander

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 known to be or which might potentially be infested with the relevant pathogens.

Coriander seed testing

Seeds were tested according to a method 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 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 & Roberts 1995).

Coriander seed transmission

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 & 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.

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 then vent at 20/17°C. Temperature was monitored continuously using a Tinytag temperature logger. Two 308 trays were sown for each inoculum dose and method. In addition, one tray was 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 (*Psc*). 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 sizes 2, 2, 2, 14, 44, and 70 plants 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%. 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 in 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.

Statistical analyses

The proportions of infested seeds in the naturally infested seed lots and their 95% confidence limits were estimated by maximum likelihood methods using the STPro™ seed test analysis program (Ridout & Roberts 1995). The mean numbers of bacteria on the naturally infested seed 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 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).

Results

Coriander seed testing

Six out of the nine 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 Table 1, 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 35 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	<0.02	0.00	0.02	<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}

Coriander Seed Transmission

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. Contaminated seedlings were detected only for the two highest doses. The proportion of seedlings contaminated was estimated using the STPro program and results are shown in Table 2.

As the two doses where transmission was detected were relatively close together it was not possible to fit a meaningful model to the data. The one-hit probability of transmission was therefore calculated separately for each of these treatments to provide a mean estimate of the one-hit probability of transmission of 3.6×10^{-7}.

Discussion

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 also bring into question the reliability of the test results from some well-known 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 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 is to estimate this 'one-hit' probability.

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				
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
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 coriander transmission experiment used both naturally infested and artificially inoculated seed to look at dose/response relationships, i.e. the relationship between the

numbers of bacteria on the seed and the proportion of plants emerging which are contaminated. Transmission occurred at a lower frequency than expected and was only detected at the highest inoculum levels. Although a value for the 'one-hit' transmission was obtained, this cannot be considered to be a robust estimate, as the form of the dose/response relationship (i.e. the model) means that the data is relatively uninformative when all units are negative (i.e. when transmission is not detected). An additional transmission experiment is now underway with a higher dose than in the previous experiment.

Developing appropriate seed health standards for parsley

Objective

The objective was to determine for individual parsley seed lots:

- 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
- The frequency of transmission of *Septoria petroselini* from parsley seed to seedlings

Methods

Seed testing

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 & Kongsdal, 2003) was taken. From the sample, four sub-samples of 100 seed were taken at random (using the 'hand-halving method'; Mathur & 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 (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 40°0 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 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 & Kongsdal (2003). Parsley seed from the sample was sown in six modified module trays (204 modules per tray) in F1 compost, 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 checked 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 checked 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 & 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.

Statistical analyses

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.

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 3, standard deviations shown in Appendix 1). 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 3. 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 4). 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 2 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 4. 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

For the single seed lot (code G) for which Septoria transmission was demonstrated, transmission is being studied further, using a second method. Seeds (six replicates of 150) are being germinated in filter paper pleats and subsequently exposed to leaf wetness periods of at least 72 h, before being monitored for lesion development. Results will be presented in the next Annual report.

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 no 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. The transmission rate for *S. petroselini* from seed to seedling was estimated using a single seed lot. To further verify this rate, and to investigate further the relative contributions of seeds with and without pycnidia, seed lots with different mean doses of viable spores would be required.

Alternative methods for treatment of coriander and parsley seed, for control of coriander bacterial blight and parsley Septoria.

Introduction

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

Coriander

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

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 are not considered worth pursuing further.

Recent work done in Australia (Dennis and Wilson 1997; Hooper and Dennis 2002) suggests 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 is not considered to be worth pursuing further.

In a recent EC-funded project (STOVE) to examine organically acceptable treatment 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 is 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. Facilities are not currently 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 seedborne 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 seedborne 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. It is currently being investigated in another HDC project (FV 335) and depending on results may be worth evaluating as a seed treatment.

Parsley

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 STOVE project 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*. In four field trials, seed treatments with hot water, aerated steam, BA 2552 (*Pseudomonas chlororaphis*), thyme oil, and the combination of aerated steam and BA 2552 reduced

the disease to a similar extent as the chemical thiram. Treatment with *Bacillus subtilis* K 3 also showed good results. These treatments also had positive effects on yield. In the parsley / *Septoria* pathosystem it could not be determined whether combination treatments of physical and biological methods were better than a single treatment.

In HDC project FV 237a, a range of seed treatments were evaluated for control of a similar pathogen, *Septoria apiicola* on celery seed. Hot water treatment (48°C, 30 min) without a pre-soak, reduced *S. apiicola* spore germination to 3% (compared with 34% in the untreated control), without affecting seed germination either immediately after treatment, or 8 months after storage. Similar conditions are now used by industry for treatment of organic celery seed. It was found that a pre-soak prior to hot water treatment could increase the efficacy of pathogen kill, but that for some seed batches there were deleterious effects on seed germination. For example, pre-soaking celery seed for 1-6 hours prior to hot water treatment (48°C, 30 minutes) did not reduce percentage seed germination, but for one of two seed batches, a pre-soak of 24 hours reduced percentage germination compared to the hot water treatment only. In agreement with STOVE results, seed batches tested varied in their sensitivity to hot water treatment, due probably to factors such as seed age or levels of infection, or variability in seed maturity which can be significant in Umbelliferae. Protocols developed for hot water treatment on a commercial scale should take account of this variability.

In FV 237a, promising results were obtained with Jet 5 (5% peroxyacetic acid), both as a soak and also as a vapour treatment for *S. apiicola* on celery seed, although further studies would be needed to optimise application rates and treatment durations, to ensure that seed germination was not affected. For example, 20% Jet 5, reduced *S. apiicola* spore germination to 0.1%, but had a deleterious effect on seed germination after storage (4 months). Since there is no approval for use of peroxyacetic acid on seed at present, efficacy tests using this disinfectant against *S. petroselini* on parsley seed will not be undertaken within this project.

A range of other physical and biological treatments were tested in FV 237a. Treatment with UV-A, UV-B and UV-C had negligible effect on Septoria levels or celery seed germination, due largely to UV absorption by seed pigments. Microwave treatments of 120 seconds or more reduced *S. apiicola* spore germination to approximately 10% but also had deleterious effects on seed vigour. The essential oils (pine, *Eucalyptus citriadorus* and winter savory) used both in the liquid and vapour phase, and the biological control agent (Polyversum®) used in these studies were ineffective in reducing seed-borne *S. apiicola*.

Discussion

Several potential seed treatments have been identified and these will be examined in Year 2 of the project. These will include: hot water, thyme oil, at least one biological (e.g. Serenade™) and one conventional disinfectant (chlorine dioxide). However it is important to be clear that it is unlikely that we will be able to obtain complete eradication with any treatment.

Identify potential forecasting approaches for parsley Septoria

In order to meet consumer demands, growers need to minimise fungicide use while still producing high quality crops. Knowledge of environmental conditions that are favourable or unfavourable for the development of parsley Septoria can help to minimise spray applications. Forecasting models are available based on temperature and leaf wetness duration that could potentially be validated or used as a basis for a simple decision-tree as an aid to spray timing for management of parsley Septoria.

The effect of inoculum concentration, leaf age, temperature, and leaf wetness duration on *Septoria petroselini* infection and the development of Septoria blight of parsley were studied under controlled environment conditions by Kurt & Mehmet Tok (2005). Their results provide a useful summary of factors affecting the development of parsley Septoria. A minimum inoculum concentration of 10^4 conidia ml^{-1} of *S. petroselini* was required for disease development. The mean number of lesions per unit leaf area increased with inoculum concentrations from 10^4 to 2×10^6 conidia ml^{-1}. As leaves of parsley plants matured, they became more susceptible to *S. petroselini* infection. When parsley plants were sprayed, inoculated with conidial suspensions of *S. petroselini*, exposed to leaf wetness durations of 0, 12, 24, 48, 60, and 72 h, then incubated for 3 weeks at 15, 17, 20, 23, 27, and 32°C, Septoria symptoms developed at all temperatures tested. At 20 and 23°C, the mean number of lesions per plant increased as wetness duration increased at 20, whereas lesion numbers decreased as wetness duration increased at temperatures of 15, 17, 27, and 32°C. The optimum temperatures for lesion development were 20 and 23°C. At those temperatures, the optimum leaf wetness duration was 72 h. Leaf wetness duration did not contribute to an increase in disease severity at 15, 17, 27, and 32°C. Leaf area covered by pycnidia increased in treatments at temperatures between 17 and 23°C following a wetness period of 48 h. Low levels of Septoria blight on parsley developed across a wide temperature range. Leaf wetness duration significantly increased disease severity, but only at optimal temperatures.

Krauthausen *et al.* (2001) showed that after inoculation of parsley plants and a period of high humidity, first symptoms developed after 9 days at a constant temperature of 25°C. Under fluctuating temperatures (24°C day/18°C night) disease severity was higher than at constant temperatures.

There are no models that have been developed specifically for predicting the development of Septoria on parsley. However, there are three models that have been developed or adapted for the prediction of the closely related disease, Septoria late blight on celery (*S. apiicola*) and one model that has been developed for early blight of celery (*Cercospora apii*)

Model 1: Lacy model

This was developed by Lacy (1994) for celery Septoria and involves use of a leaf wetness sensor placed within the crop row at a height of 0.3 m. The prediction of infection risk is based on 12 hours or longer of leaf wetness. According to the model, the first treatment is initiated after 12 or more hours of continuous leaf wetness. The timing of subsequent treatments is also based on 12 or more hours of leaf wetness, after a minimum of a seven-day spray interval.

The model does not include temperature as an input variable, because temperature was not a limiting factor for disease development in Michigan, where the model was developed. There has been limited implementation of this model.

Model 2: Mathieu & Kushalappa model

The model developed by Mathieu & Kushalappa (1993) for celery Septoria uses air temperature and leaf wetness duration as the input variables. It predicts the proportion of the maximum number of lesions (PML) as a function of duration of leaf wetness and temperature during wet periods. Disease severity values (DSVs) were then calculated from predicted PML values using cluster analysis. Accumulation of DSVs begins when celery transplants have recovered from transplant shock. After canopy closure, accumulation of DSVs ends, and the model reverts to a weekly application of treatments. Action thresholds have not been developed for this model and implementation has been limited.

Model 3: Tom-Cast

This model was originally developed as FAST, a forecast system for *Alternaria solani* on tomato (Madden *et al.*, 1978) but was then simplified into Tom-Cast (Pitblado, 1992) for other tomato diseases. The model has subsequently been implemented for prediction in a range of crop/disease situations, including celery Septoria (Bounds &

Hausbeck, 2007; Raid *et al.*, 2007) and asparagus Stemphylium (Meyer *et al.*, 2000). The system utilises the duration of leaf wetness and average temperature during the wetness period to calculate a daily disease severity value (DSV). A fungicide is applied when the cumulative DSV reaches a predetermined threshold. The model has been validated for celery Septoria in Michigan state and Florida USA, and has also been introduced to celery growers in Victoria, Australia. Data can be downloaded from a temperature/leaf wetness sensor in the canopy and run through the Tom-Cast programme to provide a spray decision.

Model 4: Berger model

This model was developed by Berger (1969, 1973) for *C. apii* on celery using leaf wetness and relative humidity data, which was correlated in early work with counts of airborne conidia of *C. apii* within and near celery fields. In addition to monitoring of temperature and relative humidity, the Berger model requires use of a rather complex five-stage decision tree that could be off-putting to growers (Raid *et al.*, 2008).

Raid *et al.* (2008) evaluated the Berger model and Tom-Cast for scheduling sprays for management of early blight of celery (*C. apii*). Excellent control of early blight was achieved in two seasons using the Tom-cast and the Berger models with three to four sprays compared with 13 sprays applied on a weekly, calendar basis. In a third season, the Berger model was most effective. Bounds & Hausbeck (2007) evaluated the Lacy model, Tom-cast (using three different thresholds) and the Berger model for scheduling sprays for management of late blight of celery (*S. apiicola*). The Tom-Cast 10-DSV predictor resulted in disease control comparable with 7-day interval sprays but required up to five fewer sprays. The Lacy and Berger models, and Tom-Cast using 15 and 20-DSV predictors often provided the same level of control as the weekly spray, but were less consistent than Tom-Cast at 10-DSV.

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. Use of Tom-cast for scheduling sprays for control of parsley Septoria will be trialled in project year 3.

Evaluation of fungicides for control of parsley Septoria

Introduction

The objective was to evaluate in a replicated, inoculated pot experiment the relative protectant and curative activity of approved and novel fungicides applied at specified intervals before and after an infection event, for the control of parsley leaf spot

Methods

The experiment comprised a two-way factorial design with fifteen plants per plot and four replicate blocks. There were eight fungicide treatments applied at four different timings, with a full replication of the inoculated water-only control for each timing, to give a total of 36 treatments and 144 plots. A plot comprised a half-size seed tray containing fifteen plants (three rows of five), artificially inoculated with *Septoria petroselini*. Four extra trays (each with fifteen plants) were placed at least 5 m away from the main trial area (to avoid infection via spore splash) as uninoculated untreated controls (not included in statistical analysis).

Parsley seeds (*Petroselinum hortense*, flat leaf variety) were sown in F1 compost in ½ size seed trays, with 15 seeds per tray (3 rows of 5). Sowing was done on 20 July 2007. The experiment was sited on a hard-standing area within rabbit fencing. The trays were placed in raised chitting trays containing capillary matting, covered with crop mesh to minimise pest damage. The trays were overhead watered as required to maintain moist but not water-logged compost. The trays were maintained until plants reached the 4 true-leaf stage (approximately 5 weeks after planting), and gapped-up using spare plants

Fresh and 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 was filtered through sterile muslin. The final concentration of the spore suspension was 2×10^4 spores/ml.

To test inoculum viability, 50 µl spore suspension was pipetted onto each of three plates of PDA+S. Percentage spore germination was checked after incubation for 16 h at approximately 20°C.

Plants to be inoculated were sprayed to run-off using a pump action sprayer then covered with polythene until the next fungicide application was due (at least 24 h). The uninoculated control trays were sprayed with water only and covered for the same period as the test plants using a separate covering.

Fungicide treatments were applied either 3 days before, 1 day before, 1 day after or 3 days after artificial inoculation according to the treatment list in Table 5. 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.

The seed trays were subsequently overhead watered as necessary to maintain moist but not water-logged compost.

The plants were assessed 10, 17 and 27 days after inoculation, recording for each tray the number of plants (out of 15) with symptoms of Septoria leaf spot and the severity of symptoms (percent leaf area affected) on each plant. Each plot was checked for the presence or absence of phytotoxic symptoms or growth benefits.

The experiment was repeated, commencing from 4 October 2007. In the repeated experiment, curly leaf parsley (*P. crispum* var. Parsley Bravour) was used. Seeds were sown in FS2 compost in module trays. Seedlings were subsequently transferred to M3 compost in half-seed trays (15 per tray) to use for the experiment. Because of cooler weather, the experiment was done in a glasshouse rather than on the hard standing area. The fungicide treatments were as described for the first experiment. Inoculum was again prepared from fresh and dried leaves of parsley with typical symptoms of parsley leaf spot. In this experiment, inoculum was applied at a higher spore concentration of 1.5 x 10^5 spores/ml. Assessments were done as for the first experiment.

Table 5. Fungicide treatments evaluated against parsley Septoria

	Product	Active ingredient	Product rate
1	Untreated control	-	-
2	Amistar	Azoxystrobin	1 L/ha
3	Signum	Boscalid + pyraclostrobin	1.5 kg/ha
4	Scotts Octave	Prochloraz	0.2 kg/ha
5	Karamate Dry Flo Newtec	Mancozeb	3.9 kg/ha
6	Folicur	Tebuconazole	0.75 L/ha
7	Headland Inorganic Liquid Copper	Copper oxychloride	4.0 L/ha
8	Switch	Cyprodinil + fludioxonil	0.8 kg/ha
9	Amistar Top	Azoxystrobin + difenoconazole	1 L/ha

Notes:
Amistar – SOLA 1293/02
Signum – SOLA 1984/04
Octave – SOLA 0650/01
Karamate Dry Flo Newtec – SOLA 1978/06
Folicur – SOLA 1873/03

Headland Inorganic Liquid Copper – SOLA 1057/05
Switch – SOLA 2079/07
Amistar Top – Administrative Experimental Approval (use rate from SOLA 1476/06 on parsley root)

Results and discussion

In both experiments, the viability of the spores applied as inoculum was confirmed (>75% spore germination on agar). There were no phytotoxic effects or growth benefits following application of any of the fungicides to parsley in either of the experiments.

The efficacy of the products against parsley Septoria could not be assessed because there was nil development of Septoria on any of the parsley plants. The experiment will be repeated in 2008, using modified conditions to increase the chance of disease development within experimental plots. Possible modifications include use of a higher concentration of spores and a longer period of leaf wetness following inoculation.

Conclusions

Coriander seed testing

- A significant proportion of coriander seed lots tested were infested with *Pseudomonas syringae* pv. *coriandricola* (*Psc*)
- Growers should ideally only use coriander seed which has been tested for *Psc* to a known tolerance standard.
- Seed health test results for *Pseudomonas syringae* pv. *coriandricola* obtained by some laboratories may be of doubtful validity.

Coriander seed transmission

First estimates were obtained for seed to seedling transmission probabilities for coriander/bacterial blight.

Parsley seed testing

Tests with parsley seed lots infested with *Septoria petroselini* demonstrated that neither the percentage of seeds with spore cases nor the percentage of seeds showing spore release provides a reliable measure of pathogen viability or subsequent disease risk to the crop.

Parsley seed transmission

First estimates were obtained for seed to seedling transmission probabilities for parsley Septoria.

Alternative seed treatment methods

Several potential seed treatments have been identified and these will be examined in Year 2 of the project. These will include: hot water, thyme oil, at least one biological (e.g. Serenade™) and one conventional disinfectant (chlorine dioxide).

Potential forecasting approaches

Of the 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. It requires relatively inexpensive equipment, provides a straightforward output and has been implemented by growers. Use of Tom-cast for scheduling sprays for control of parsley Septoria will be trialled in project year 3.

Evaluation of fungicides for control of parsley Septoria

In two pot experiments, the efficacy of fungicides products against parsley Septoria could not be assessed because there was nil development of Septoria on any of the parsley plants. The experiment will be repeated in 2008, using modified conditions to increase the chance of disease development within experimental plots.

Acknowledgements

We thank seed companies who provided seed lots for experimental work.

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Appendices

Appendix 1.

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

Appendix 2

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**