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 2009
Previous report	Annual report, 2008
Key staff:	Dr S J Roberts, Plant Health Solutions – Coriander Ms Angela Huckle, ADAS
Location of project:	ADAS Arthur Rickwood and Boxworth, Cambridgeshire; Plant Health Solutions, Warwick; HDRA, Ryton, Coventry
Project coordinator:	Dan Nye, CN Seeds Ltd, Denmark House, Pymoor, Ely, Cambridgeshire.
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, epidemiology

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.

Dr K R Green
Research Scientist
ADAS

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

Dr S J Roberts
Director
Plant Health Solutions

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

Report authorised by:

Dr W E Parker
Horticulture Research and Consultancy Manager
ADAS

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

Dr S J Roberts
Director
Plant Health Solutions

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

Table of Contents

Grower Summary

Headline

- In a coriander bacterial blight spread trial, around 10% of the crop was affected from a primary infection level equivalent to transmission by 1 in 15,000 seeds. Hot water looks to be the most promising seed treatment option for coriander bacterial blight; useful reductions were also obtained with thyme oil and biological control agents.

- Amistar (azoxystrobin), Signum (boscalid + pyraclostrobin) and Karamate Dry Flo Newtec (mancozeb) were effective as foliar fungicides for control of Septoria leaf spot on parsley. Amistar and Signum significantly reduced spore germination of *Septoria petroselini* when applied to lesions 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 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 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

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

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 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 previous coriander 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 seedlots 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 (See Table 1). Examination of the scenarios in the table suggests that an appropriate testing scheme could be effective in reducing the

Table 1. Some example scenarios for expected transmission in a block sown with 1,000,000 coriander seeds (fruits), equivalent to approx. 0.36 ha and two different seed testing schemes.

1 inf seed in:	% inf	CFU per inf seed	Pr. transmission [1]	Pr. detection[2] in seed test on:	
				1 x 3k	3 x 5k
15,000	0.007	100	0.038	0.05	0.11
15,000	0.007	1,000	0.072	0.17	0.55
15,000	0.007	10,000	0.133	0.18	0.63
15,000	0.007	100,000	0.240	0.18	0.63
10,000	0.010	100	0.057	0.07	0.14
10,000	0.010	1,000	0.106	0.25	0.67
10,000	0.010	10,000	0.193	0.26	0.78
10,000	0.010	100,000	0.337	0.26	0.78
5,000	0.020	100	0.111	0.13	0.17
5,000	0.020	1,000	0.201	0.44	0.82
5,000	0.020	10,000	0.349	0.45	0.95
5,000	0.020	100,000	0.561	0.45	0.95
1,500	0.067	100	0.324	0.25	0.18
1,500	0.067	1,000	0.527	0.83	0.86
1,500	0.067	10,000	0.761	0.86	1.00
1,500	0.067	100,000	0.936	0.86	1.00
1,000	0.100	100	0.444	0.27	0.18
1,000	0.100	1,000	0.674	0.92	0.86
1,000	0.100	10,000	0.883	0.95	1.00
1,000	0.100	100,000	0.984	0.95	1.00

[1] Probability of at least one infected seedling in the block.
[2] Probability of detection in seed test performed according to PHS standard method.

prevalence of coriander bacterial blight.

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. Field trials were done on the organic land at Ryton (Garden Organic/HDRA). Plots consisting of 3 (5-row beds) x 10 m were drilled on two occasions with healthy coriander seed. 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.

The first drilling provided some useful data and a mathematical model was successfully fitted (Figure 1). These model parameters will assist in defining appropriate seed health standards. At the time of the final recording when the crop was in flower, up to 10% of the crop was affected following a primary infection level equivalent to transmission by 1 in 15,000 seeds (fruits).

Unfortunately due to delays in drilling (due to wet weather in August 2008), the second crop provided little useful data.

The trials will be repeated in Year 3, and the results from the first trial indicate that there is no need to change the approach.

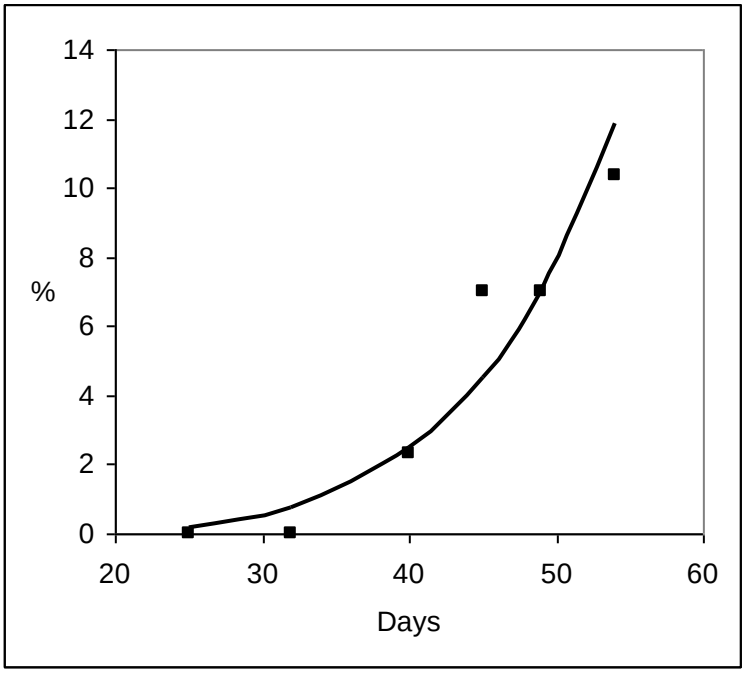

Figure 1. Change in the percentage of quadrats with bacterial blight symptoms in a field plot (10 m x 3 beds) of coriander with a single central point source of inoculum. The line represents the fitted model.

Coriander bacterial blight seed treatment

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

Table 2. 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 standard errors.

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 results suggest that hot water is the most promising treatment and is

worthy of more detailed investigation of treatment parameters, and with more seed lots, in the final year of the project. All hot water treatments gave very significant reductions in *Psc*. The best treatment, 55°C for 15 min, reduced *Psc* numbers to undetectable levels in the naturally infested seedlot examined, without any reduction in germination. Thyme oil also gave a significant reduction and may be worthy of continuing investigation, but most surprisingly 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 (Table 3). This is also consistent with the results obtained in FV 335 (Roberts 2009) for another bacterial disease (black rot of brassicas).

Table 3. Summary of transmission studies on two bacterial blight infested coriander seedlots 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 Max	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 Max	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 standard errors

Fungicides for parsley Septoria

Experiments using curly leaf parsley in trays or pots in a glasshouse were done to:

- Evaluate 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.
- Determine the effects of fungicides applied after lesion and pycnidial development had occurred on parsley leaves inoculated with *Septoria petroselini.*

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 (Figure 2).

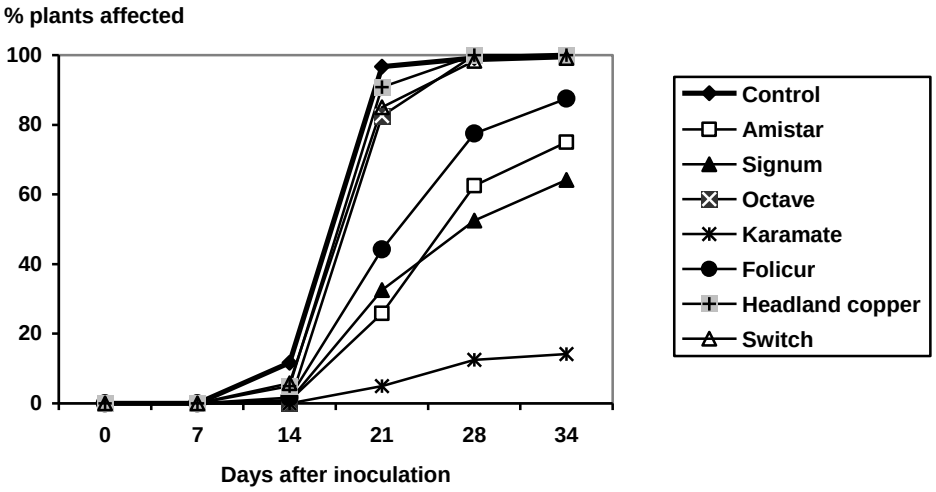

Figure 2. Incidence of Septoria leaf spot (*Septoria petroselini*) on parsley following fungicide applications, with data averaged across timings

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, while Karamate gave excellent control even when applied 5 days before inoculation.

When the same range of fungicides was 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 (Figure 3). This mode of action could be useful in controlling a disease such as parsley leaf spot which is polycyclic, i.e. having many disease cycles within a season. Application of a strobilurin product to mature Septoria lesions will not necessarily affect development of the fungus

in the plant, but could at least limit secondary spread of the disease by limiting germination of spores present in pycnidia.

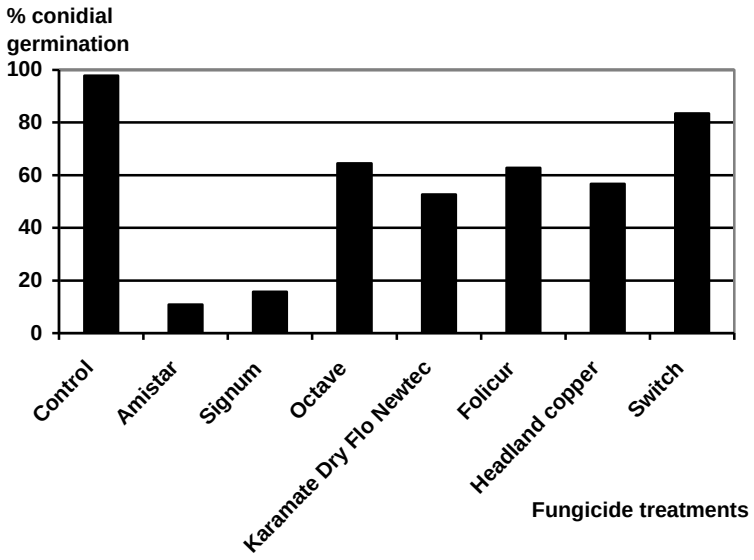

Figure 3. Effect of fungicide treatments applied to lesions of parsley leaf spot, on the germination of conidia of *Septoria petroselini*

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.

- The following fungicides are permitted for use on outdoor parsley and are effective against parsley leaf spot (*Septoria petroselini*). Follow SOLA conditions of use.

Amistar	Azoxystrobin	SOLA 1293/02
Signum	Boscalid + pyraclostrobin	SOLA 1984/04
Scotts Octave	Prochloraz	SOLA 0650/01
Karamate Dry Flo Newtec	Mancozeb	SOLA 1978/06

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 fact sheet on integrated strategies for management of parsley Septoria and coriander leaf blight

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

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

Developing appropriate seed health standards for coriander

Introduction

The transmission experiment done in the first year was partially repeated (with the highest doses) in order to improve the reliability of the estimated 'one-hit' transmission probability.

'Healthy' seedlots identified in Year 1 were no longer available, therefore it was necessary to obtain and test further seedlots for use in the field trials.

Trials to provide data on the rate of disease spread in the field were done. Following discussions with the grower coordinator it was decided to drill on two separate dates and irrigate all crops as necessary rather than as originally proposed to have two simultaneously drilled plots with/without irrigation.

Materials and Methods

Source of seeds

Contacts were made with a number of seed companies supplying coriander seed and requests made for further samples of seed lots identified as being useful to the project (either because of apparent freedom from disease or with high levels of infestation).

Coriander seed testing

Seeds were tested according to the methods developed by Plant Health Solutions for commercial routine testing of coriander seed for *Psc*, as described in the Year 1 report (Green and Roberts 2008). Infestation levels were quantified by testing repeated sub-samples of seed of varying sizes for each seed lot, as described in the Year 1 report.

Coriander seed transmission

Seed samples that had been vacuum inoculated with the three highest doses of *Psc* (isolate 9021, seed lot S1046) in Year 1 were used.

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. Two 308 trays were sown for each inoculum dose.

Assessment

Rather than waiting for symptom expression, transmission was estimated by determining the proportion of seedlings contaminated with the pathogen (*Psc*), as in Year 1. 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 varying from 7 to 50 cells 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 0.1% to 20% for the two lowest doses and 1% to 40% for the highest dose. 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.

Samples were processed by stomaching, diluting and plating on selective media, as described in Year 1.

Spread in the field

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

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 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.

The first trial was drilled on 27 June 2008 and the second was drilled on 29 August 2008.

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.

Results

Coriander Seed Transmission

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 1.

Table 1. Relationship between mean dose of bacteria per seed and transmission from seed to seedling in a second experiment, for coriander seeds inoculated with *Pseudomonas syringae* pv. *coriandricola.*

Treat. Code	Dose (CFU/seed)	% Transmission	95% confidence limits	
			Lower	Upper
Vacuum inoculated				
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

These new data were then combined with the transmission data from the experiment done in Year 1 in order to obtain an improved 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.

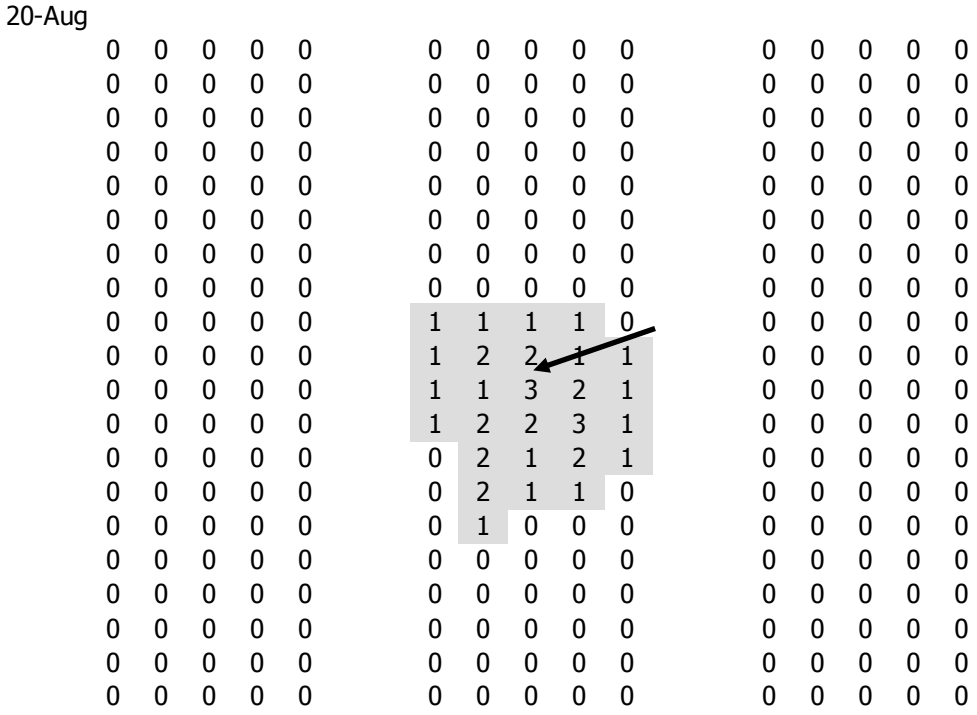

Figure 1. Map showing the distribution of bacterial blight symptoms, 54 days after sowing in coriander field plot (10 m x 3 5-row beds) to examine the spread of *Pseudomonas syringae* pv. *coriandricola.* The arrow indicates the position of the primary infector plants. Each number represents the disease severity score (0-4 scale) in a 0.5 m section of row

Spread in the field

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 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.

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.

A generalised non-linear model was fitted to the symptom data for the first spread trial. The model was in the form:

$$\text{logit}(p) = a - b\ln[(c + kx^2 + y^2)^{½}] + rt$$

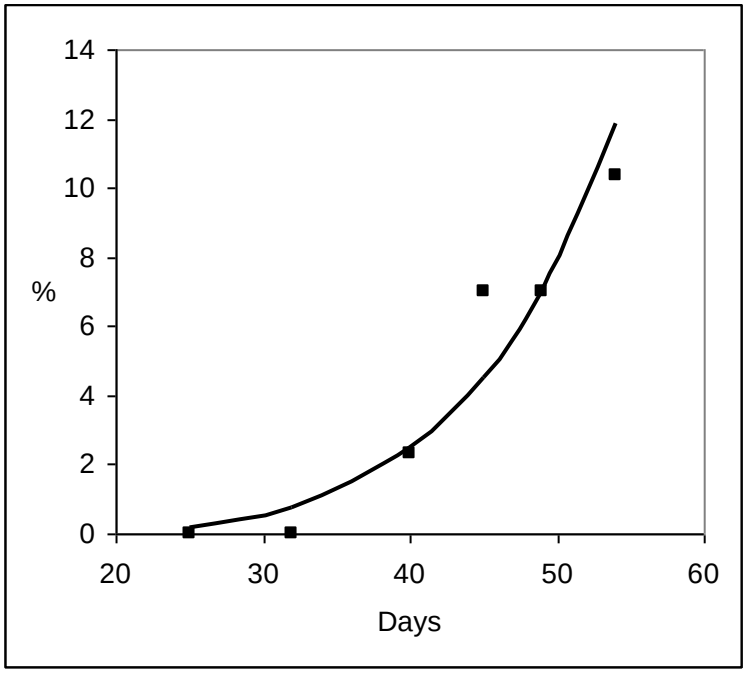

Figure 2. Change in the percentage of quadrats with bacterial blight symptoms in a field plot (10 m x 3 beds) of coriander with a single central point source of inoculum. The line represents the fitted model.

where p is the proportion of quadrats with symptoms, a is an intercept parameter, b is the disease gradient, x and y are distances from the source (primary infector) in the between and within row directions, k is a dimensional scaling parameter to account for different rates of spread in the x and y directions, 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 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 0.25 (half the length of a quadrat), and the model was specified with a binomial error distribution and logit link function. Parameter estimates and their standard errors are given in Table 2

Table 2. Parameter estimates for model fitted to data from first coriander/bacterial blight spread trial.

Parameter	estimate	s.e.
k	1.25	0.35
a	-11.6	1.96

b	-4.79	0.54
r	0.257	0.043

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

Discussion

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 is to estimate this 'one-hit' probability.

The previous coriander 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 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 can be used to predict the likelihood of disease transmission for seedlots with different levels of infestation and examine these values in relation to the probability of detection for different seed health testing schemes (See Table 3)

Field Trial

The first field trial to examine spread of coriander bacterial blight from a point source provided some useful data and a mathematical model was successfully fitted. At the time of the final recording when the crop was in flower, up to 10% of the crop was affected following a primary infection level equivalent to transmission by 1 in 15,000 seeds (fruits). No spread was detected between beds, but it is possible that this was an artefact of the orientation of the crop in relation to the prevailing weather during spread events. Unfortunately the second trial did not provide any useful data due to the delayed drilling, and therefore as the model parameters are based on only a single dataset it would be premature to make too much of them at this stage.

The trials will be repeated in Year 3, and the results from the first trial indicate that there is no need to change the approach. However, to avoid the problems of last year with the later crop, it is proposed that both crops will be drilled slightly earlier.

Table 3. Some example scenarios for expected transmission in a block sown with 1,000,000 coriander seeds (fruits), equivalent to approx. 0.36 ha and two different seed testing schemes.

1 inf seed in:	% inf	CFU per inf seed	Pr. transmission [1]	Pr. detection[2] in seed test on:	
				1 x 3k	3 x 5k
15,000	0.007	100	0.038	0.05	0.11
15,000	0.007	1,000	0.072	0.17	0.55
15,000	0.007	10,000	0.133	0.18	0.63
15,000	0.007	100,000	0.240	0.18	0.63
10,000	0.010	100	0.057	0.07	0.14
10,000	0.010	1,000	0.106	0.25	0.67
10,000	0.010	10,000	0.193	0.26	0.78
10,000	0.010	100,000	0.337	0.26	0.78
5,000	0.020	100	0.111	0.13	0.17
5,000	0.020	1,000	0.201	0.44	0.82
5,000	0.020	10,000	0.349	0.45	0.95
5,000	0.020	100,000	0.561	0.45	0.95
1,500	0.067	100	0.324	0.25	0.18
1,500	0.067	1,000	0.527	0.83	0.86
1,500	0.067	10,000	0.761	0.86	1.00
1,500	0.067	100,000	0.936	0.86	1.00
1,000	0.100	100	0.444	0.27	0.18
1,000	0.100	1,000	0.674	0.92	0.86
1,000	0.100	10,000	0.883	0.95	1.00
1,000	0.100	100,000	0.984	0.95	1.00

[1] Probability of at least one infected seedling in the block.
[2] Probability of detection in seed test performed according to PHS standard method.

Alternative seed treatment methods for control of bacterial blight

Introduction

The potential seed treatments identified in Year 1 were examined. These included: hot water, thyme oil, two biological control agents (BCAs) (Subtilex™ and Serenade Max™) and one conventional disinfectant (chlorine dioxide).

Materials and Methods

Seed

Further larger quantities of 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 seedlot (S1081).

Seed Treatment

Hot water treatment was 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.

Thyme oil treatment was done with a 10% oil/water emulsion, prepared by sonication and stabilised using 0.1% agar. 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 of the above 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) 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 each (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. daylength 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% isopropanol.

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 generalise linear model using the Genstat statistical analysis program (Payne *et al.* 2005).

Results

Physical/chemical treatments

In initial tests, using naturally infested seedlot 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 Table 4). Further testing/re-testing of stocks of infested seedlots was therefore necessary to identify an alternative seed lot for use in the treatment studies, and subsequently evaluation was done with seedlot S1072.

The total % germination following treatment is shown in Table 4. 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 4. Germination in two coriander seedlots 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 naturally infested seed lot S1072 are summarised in Table 5. 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 5. 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.

Biologicals

Results of the transmission studies in seed treated with BCAs are shown in Table 6.

Table 6. Summary of transmission studies on two bacterial blight infested coriander seedlots 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

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 seedlot.

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 seedlot 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 seedlot.

Seedlot S1045 had been selected for treatment work because previous 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 two possible explanations for this difference:

1. The seedlot 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 seedlots 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 results of seed tests on physically/chemically treated coriander seeds suggest that hot water is the most promising treatment and is worthy of more detailed investigation of treatment parameters, and with more seed lots, in the final year of the project. All hot water treatments gave very significant reductions in *Psc*. The best treatment, 55°C for 15 min, reduced *Psc* numbers to undetectable levels in the naturally infested seedlot examined, without any reduction in germination. Thyme oil also gave a significant reduction and may be worthy of continuing investigation, but most surprisingly 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).

Evaluation of fungicides for control of parsley Septoria

Introduction

The objectives were to:

- Evaluate 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.
- Determine the effects of fungicides applied after lesion and pycnidial development has occurred on parsley leaves inoculated with *Septoria petroselini.*

Methods

Experiment 1

Parsley seeds (*Petroselinum crispum*, var. Bravour, curly leaf variety) were sown in F1 compost in module trays (1 seed per cell, approx. 1300 seedlings) (3 October 2008). The module trays were placed on damp capillary matting in a glasshouse at 20^{o}C day / 15^{o}C night with supplementary lighting (12 h day / 12 h night). Once seedlings had reached 2-3 true leaves (approx. 4 weeks after sowing), they were transplanted to ½ size seed trays, with 10 seeds per tray (2 rows of 5).

The experiment was sited in a heated glasshouse at ADAS Arthur Rickwood, Cambridgeshire and comprised a two-way factorial design with ten plants per plot and four replicate blocks. There were seven fungicide treatments applied at three different timings, with a full replication of the inoculated water-only control for each timing, to give a total of 24 treatments and 96 plots. A plot comprised a half-size seed tray containing ten plants, artificially inoculated with *Septoria petroselini*. Four extra trays (each with ten plants) were placed in an adjacent glasshouse compartment (to avoid infection via spore splash) as uninoculated untreated controls (not included in statistical analyses). The trays were placed in raised chitting trays containing capillary matting and were overhead watered as required to maintain moist but not water-logged compost. The trays were maintained for a further 4 weeks until plants reached at least 6 true leaves.

A series of preliminary studies was done prior to this experiment to determine the most effective method for achieving consistent symptom development on parsley using artificial inoculation with *Septoria petroselini*. In project year 1, artificial inoculum was prepared using fresh and dried leaf material from parsley plants that had typical symptoms of Septoria leaf spot. Resulting infection levels were low, due probably to low spore numbers, viability, or less than optimum environmental conditions. In project year 2, it was found that using spores of *S. petroselini* from culture plates could provide high numbers of viable spores for use in artificial inoculation.

Based on findings from preliminary studies, inoculation was done in this experiment as follows: An isolate of *S. petroselini ex* parsley (culture ref: ADAS AR07/164) was sub-cultured onto 220 plates of V8 agar (200 ml V8 juice, 20 g agar and 800 ml distilled water). Plates were incubated in the dark at 20^{o}C until there was abundant sporulation (approx. 2 weeks). A spore suspension was prepared by pouring approx. 10 ml distilled water on to each plate, then dislodging spores using a sterile loop. The resulting spore suspension was filtered through muslin and spore concentration determined using a haemocytometer and microscope. The concentration of the spore suspension was adjusted to 7.5 x 10^5 spores/ml. To test inoculum viability, 50 µl spore suspension was pipetted onto each of three plates of potato dextrose agar amended with streptomycin (PDA+S). Percentage spore germination was checked after incubation for 16 h at approximately 20°C (confirmed 100% germination). The inoculum was applied to the plants to the point of run-off using a pump action sprayer, with 10 ml applied per plot. Immediately after inoculation, the plants were covered with a clear polythene 'tent', which was left in place for 48 h. The uninoculated control trays were sprayed with water only and covered for the same period as the test plants using a separate polythene covering.

Fungicide treatments were applied either 5 days before, 2 days before, or 2 days after artificial inoculation according to the treatment list in Table 7. Fungicides were applied in 1000 L water/ha (100 ml/m^2) using an Oxford precision sprayer with single nozzle at 2.5 Bar. Trays were removed to a separate glasshouse for spray treatment before being replaced in the trial.

Following artificial inoculation and fungicide treatments, watering was by hand, to the capillary matting rather than overhead watering. Integrated pest management was used as follows: *Atheta* breeding boxes were placed and maintained in the glasshouse to control sciarid flies. *Amblyseius cucumeris* was sprinkled on plants weekly during October for management of Western flower thrips.

Table 7. 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

Notes:
Amistar – SOLA 1293/02
Signum – SOLA 1984/04
Octave – SOLA 0650/01
Karamate Dry Flo Newtec – SOLA 1978/06
Folicur – previously SOLA 2040/08, now revoked
Headland Inorganic Liquid Copper – now revoked, Administrative Experimental Approval
Switch – now revoked, Administrative Experimental Approval

The plants were assessed 7, 14, 21, 28 and 34 days after inoculation, recording for each tray the number of plants (out of 10) 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.

Incidence data were analysed by fitting a series of generalised linear models (with a logit link function) in Genstat. Severity data were analysed using ANOVA (following logit transformation) in Genstat.

Experiment 2

Using plants of curly leaf parsley var. Bravour sown in module trays for Experiment 1, twenty four seedlings were transplanted to 9 cm diameter pots (3 plants per pot) at 2-3 true leaf stage. The plants were maintained on capillary matting in a glasshouse compartment (20^{o}C day /15^{o}C night) with supplementary lighting (12 h day / 12 h night) with overhead watering as necessary.

When plants had reached at least 6 true leaves, they were inoculated with *S. petroselini* using the same methods as described for Experiment 1. The plants were maintained with watering to the capillary matting, and were observed weekly for symptom development.

Once leaf lesions characteristic of Septoria leaf spot with mature pycnidia had formed, a single fungicide application was done. The treatments listed in Table 7 were each applied to three pots of parsley at the rates described. After 24 h, leaves with Septoria lesions were removed from each pot, taken to the laboratory and immersed in 50 ml sterile distilled water for 1 h. The resulting spore suspension for each pot was streaked onto a plate of PDA+S and incubated at 20^{o}C in the dark for 24 h. For each plate, percentage germination was recorded for two sets of 100 spores of *S. petroselini*. Normal germination was defined as germ tubes at least twice the length of conidia.

Data were analysed by generalised linear models in Genstat.

Results and discussion

Experiment 1

Plants in the majority of treatments showed neither phytotoxic symptoms nor growth benefits following fungicide applications. Slight symptoms of phytotoxicity (white leaf lesions) were observed in four out of the 12 plots treated with Folicur, and there were slight spray residues on plots treated with Karamate Dry Flo Newtec.

Disease development was first observed at low levels 14 days after artificial inoculation, with symptoms developing first on untreated plots, and plots treated with Headland Inorganic Liquid Copper and Switch, in at least two blocks. Disease incidence reached almost 100% by 21 days after inoculation in the untreated control (Figure 3). For plants treated with Folicur, Amistar or Signum, mean incidence remained less than 90% at 34 days after inoculation, while treatment with Karamate reduced incidence to 14%. Disease severity progress (averaged across fungicide timings) is illustrated in Figure 4, demonstrating that while severity had reached over 5% leaf area affected in the untreated control at 34 days after inoculation, treatments with either Karamate, Folicur, Amistar or Signum were effective in checking disease severity (to 1% or less).

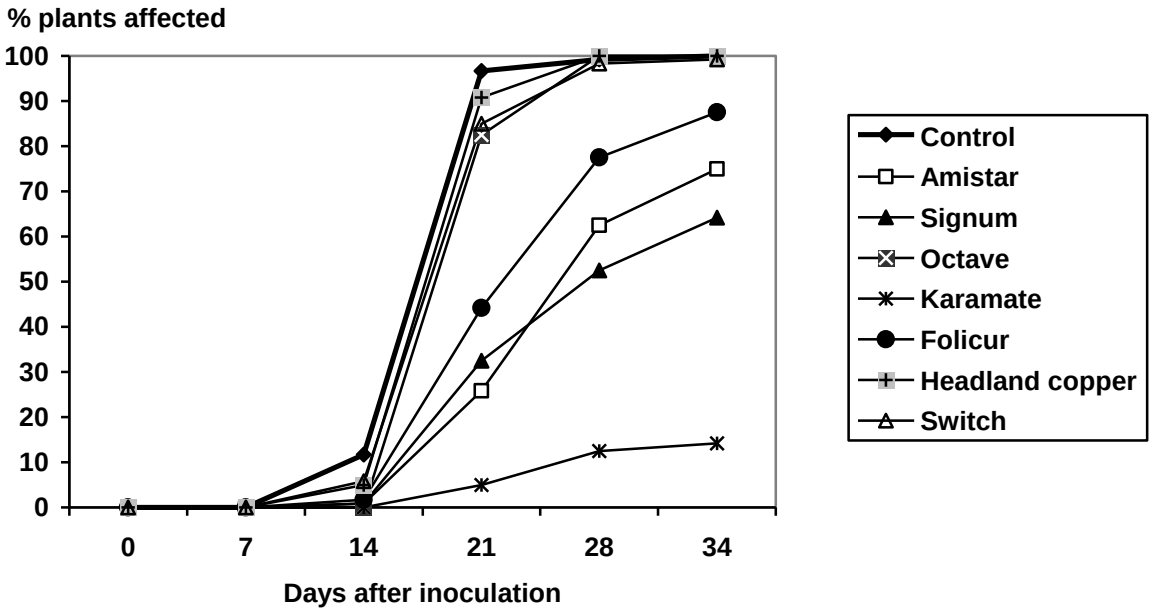

Figure 3. Incidence of Septoria leaf spot (*Septoria petroselini*) on parsley following a single fungicide application, with data averaged across timings

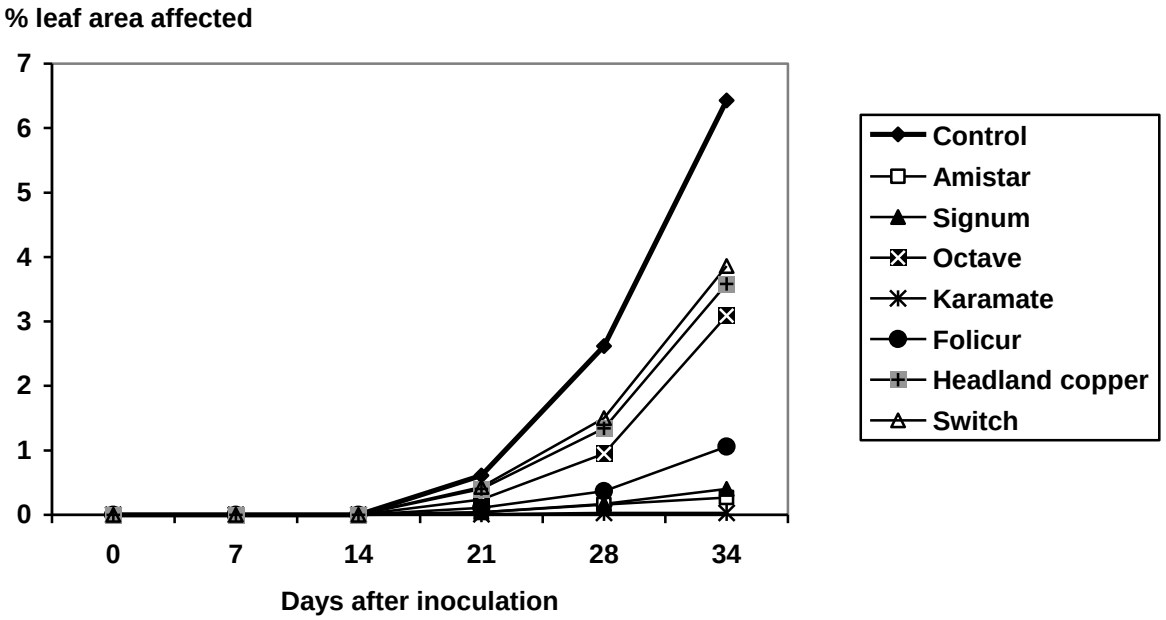

Figure 4. Severity of Septoria leaf spot (Septoria petroselini) on parsley following a single fungicide application, with data averaged across timings

Incidence and severity data for 28 days after inoculation is shown in Tables 8 and 9. At this date, there were significant differences in disease incidence between the fungicide treatments (P<0.001), and also timings (P<0.001) (Appendix 3). Amistar, Signum, Karamate Dry Flo Newtec and Folicur significantly reduced disease incidence, with Karamate being the most effective product. Overall, fungicide applications 2 days before or after inoculation were significantly more effective than the same products applied 5 days before inoculation in reducing disease incidence. However, there was a trend for an interaction effect, with Amistar apparently more effective when applied preventatively 2 days before inoculation, and disease incidence remaining low following Karamate application, irrespective of timing. For disease severity, there were significant effects of fungicide treatments, with Karamate, followed by Amistar, Signum and Folicur, again being the most effective products. There was a significant fungicide x timing interaction effect (P<0.001; Appendix 3), again demonstrating that for some fungicides (e.g. Amistar and Signum), applications 2 days either side of an infection event were most effective, while for other products such as Karamate, control was achieved even when applied 5 days prior to inoculation.

Table 8. Effect of fungicides and timing on the incidence of parsley leaf spot, 28 days after inoculation with *Septoria petroselini*.

Treatment	Mean % incidence[1] after fungicide application at different times in relation to inoculation:			Mean	±95%[2] conf. limits
	-5 days	-2 days	+2 days		

Control	99.2	99.2	99.2	99.2	-
Amistar	70.0	47.5	70.0	62.5	8.54
Signum	82.5	45.0	30.0	52.5	8.10
Scotts Octave	100.0	100.0	100.0	100.0	0.02
Karamate Dry Flo Newtec	12.5	7.5	17.5	12.5	5.96
Folicur	85.0	75.0	72.5	77.5	7.50
Headland Inorganic Liquid Copper	100.0	100.0	100.0	100.0	0.02
Switch	100.0	97.5	97.5	98.3	2.32
Mean (fungicide treatments only)	78.6	67.5	69.6		
±95% confidence limits[2]	3.45	3.95	3.98		

[1]Proportion of plants affected, ten plants per plot, four replicate blocks
[2] Approximate – non-linear model.

Table 9. Effect of fungicides and timing on the severity of parsley leaf spot, 28 days after inoculation with *Septoria petroselini*

Treatment	Mean % severity* of after fungicide application at different times in relation to inoculation			Mean
	-5 days	- 2 days	+2 days	
Control	3.3	1.4	1.7	2.61
Amistar	0.1	0.1	0.2	0.16
Signum	0.3	0.2	0.1	0.17
Scotts Octave	1.3	1.1	0.5	0.95
Karamate Dry Flo Newtec	0.0	0.0	0.0	0.03
Folicur	0.6	0.2	0.3	0.37
Headland Inorganic Liquid Copper	1.7	1.6	0.7	1.34
Switch	1.4	2.0	1.1	1.50
Mean (fungicide treatments only)	0.79	0.75	0.40	

*Percentage leaf area affected, ten plants per plot, four replicate blocks

Experiment 2

Lesions of Septoria leaf spot were confirmed on parsley plants 14 days after inoculation. However, there was nil development of pycnidia within the lesions, possibly due to difficulties in maintaining glasshouse temperatures in early January. Plants were overhead watered and re-covered in polythene for 2 days. Three days later, pycnidia containing spores of *S. petroselini* had developed within lesions and were abundant.

Spore germination in the untreated control treatment was 98% and there was a significant effect of the single fungicide treatments to Septoria lesions, on the germination of conidia (Table 10). All of the fungicides except for Switch gave a reduction in percentage spore

germination compared with the untreated control. In addition, Amistar and Signum, resulted in significantly lower percentage spore germination than the other fungicides. This finding is supported by previous work in HDC project FV 237 (Green and O'Neill 2001), in which a similar reduction in spore germination was achieved when Amistar and Signum (as coded product BAS 516 F) were applied to mature lesions of late blight on celery caused by *Septoria apiicola*. The observed effect with Amistar and Signum was initially surprising, given that strobilurin fungicides such as Amistar and Signum are routinely used for their protectant mode of action (as they are deemed most effective when applied to plant material prior to infection) rather than as curatives or eradicants. However, there are several reports in the literature demonstrating both limited curative activity of strobilurins (from translaminar activity) as well as antisporulant properties when eradicant applications are made. For example, Aseniadis *et al*. (2003) demonstrated that Amistar applied to symptoms of *Cercospora beticola* and *Erysiphae betae* on sugar beet, showed anti-sporulant activity by reducing numbers of spores produced and percentage germination. Earlier work on an experimental strobilurin formulation demonstrated inhibition of spore germination for cereal diseases such as *Puccinia recondita*, *Septoria tritici* and *Septoria nodorum* (Godwin *et al*., 1994). Since the early stages of fungal spore germination have a high energy demand, this part of the pathogen life cycle is particularly susceptible to strobilurin action which inhibits mitochondrial respiration by blocking electron transfer between cytochrome b and c. This mode of action could be useful in controlling a disease such as parsley leaf spot which is polycyclic, i.e. having many disease cycles within a season. Application of a strobilurin product to mature Septoria lesions will not necessarily affect development of the fungus in the plant, but could at least limit secondary spread of the disease by limiting germination of spores present in pycnidia.

Table 10. Effect of fungicide treatments applied to lesions of parsley leaf spot, on germination of conidia of *Septoria petroselini*

	Product	Active ingredient(s)	Mean % conidial germination [1]	95%[2] conf. limits
1	Untreated control	-	97.8	6.65
2	Amistar	Azoxystrobin	10.8	14.43
3	Signum	Boscalid + pyraclostrobin	15.7	16.88
4	Scotts Octave	Prochloraz	64.5	22.23
5	Karamate Dry Flo Newtec	Mancozeb	52.7	23.19
6	Folicur	Tebuconazole	62.8	22.45
7	Headland Inorganic Liquid Copper	Copper oxychloride	56.7	23.02
8	Switch	Cyprodinil + fludioxonil	83.5	17.23

[1]2 counts per plate; 3 replicate plates per treatment
[2]Approximate – non-linear model

Summary/Conclusions

Coriander seed testing

- Some problems encountered due to variation in infestation levels in initially selected seedlot, which necessitated re-testing of all seed stocks.
- It is vital that adequate primary samples are drawn from throughout the seed bulk when collecting samples for testing/analysis.

Coriander bacterial blight seed transmission

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

Coriander bacterial blight spread

- Bacterial blight spread from a single point source (equivalent to transmission by 1 in 15,000 seeds) to around 10% of the crop by 54 days after sowing.
- First parameter estimates have been obtained for a model describing the spread of bacterial blight in the field.

Coriander seed treatments

- Initial seed treatments and evaluations have been conducted with hot water, thyme oil, chlorine dioxide and two BCAs.
- Of the physical/chemical treatments, hot water looks to be the most promising, thyme oil also gives a useful reduction, but most surprisingly 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.

Foliar fungicides for parsley Septoria

- 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 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.

Approval status of treatments/products used

Coriander seed treatments

Hot water treatment does not require approval.

Serenade MAX (powdered formulation) 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 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.

The status of thyme oil is unclear: it does not have approval as a plant protection product in the UK, but is widely used in mouthwashes and soaps.

Foliar fungicides for parsley Septoria

The approval status of fungicides that were tested in this project year, on outdoor parsley is summarised below:

Amistar	Azoxystrobin	SOLA 1293/02
Signum	Boscalid + pyraclostrobin	SOLA 1984/04
Scotts Octave	Prochloraz	SOLA 0650/01
Karamate Dry Flo Newtec	Mancozeb	SOLA 1978/06
Folicur	Tebuconazole	No longer approved
Headland Inorganic Liquid Copper	Copper oxychloride	No longer approved
Switch	Cyprodinil + fludioxonil	No longer approved

Acknowledgements

We thank seed companies, chemical, and BCA suppliers who provided materials for experimental work.

References

Anesiadis, T., Karaoglanidis, G.S. and Tzavella-Klonari, K. (2003). Protective, curative and eradicant activity of the strobilurin fungicide azoxystrobin against *Cercospora beticola* and *Erysiphe betae*. *Journal of Phytopathology* 151, 647-651.

Godwin, J.R., Young, J.E. and Hart, C.A. (1994). ICIA5504: Effects on development of cereal pathogens. *Proceedings of the Brighton Crop Protection Conference, Pests & Diseases, 1994*. Pp 259-264.

Green, K. and O'Neill, T. (2002). *Outdoor celery: Development of integrated strategies for the management of Septoria leaf spot and other diseases*. Year 1 Report FV237. East Malling, UK: HDC. 36 pp.

Green, K. and Roberts, S. J. (2008) *Outdoor herbs: Integrated management of parsley Septoria and coriander bacterial blight. Year 1 Report FV 318*. East Malling, UK: HDC . 35 pp.

ISTA (2007) *International Rules for Seed Testing*. Bassersdorf, Switzerland: International Seed Testing Association.

Payne, R. W., Harding, S. A., Murray, D. A., Soutar, D. M., Baird, D. B., Welham, S. J., Kane, A. F., Gilmour, A. R., Thompson, R., Webster, G. and Tunnicliffe Wilson, G. (2005). *The Guide to Genstat Release 8. Part 2: Statistics*. Oxford, UK.: VSN International.

Ridout, M.S. (1995). Three-stage designs for seed testing experiments. *Applied Statistics* **44**, 153-162.

Ridout, M.S. and Roberts, S.J. (1995). STpro: Seed Test analysis program. [1.0]. HRI.

Roberts, S. J. (2009). *Evaluation of disinfectants, biological and natural products for control of brassica black rot (Xanthomonas campestris pv. campestris). Final Report 2008-09*. East Malling, UK: HDC. 38 pp.

Appendix 1. Statistical analyses – coriander seed health standards

Genstat ouptut 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

Fitted expresssion:
expr ex[1]; !e(logd = log(c + sqrt(k*xd**2+yd**2)))

Nonlinear regression analysis
==========

Response variate: symp
Binomial totals: 1
Distribution: Binomial
Link function: Logit
Nonlinear parameters: k
Model calculations: ex[1]
Fitted terms: Constant + day + logd

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio
Regression	3	398.9	132.9523	132.95
Residual	1796	255.7	0.1424	
Total	1799	654.6	0.3638	

Dispersion parameter is fixed at 1.00.

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

Estimates of parameters

Parameter	estimate	s.e.
k	1.252	0.350
* Linear		
Constant	-11.61	1.96
day	0.2574	0.0429
logd	-4.790	0.543

* MESSAGE: s.e.s are based on dispersion parameter with value 1

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. Statistical analyses – fungicides for parsley Septoria

Regression analysis (Experiment 1, disease incidence, 28 days after inoculation)

Regression analysis

Response variate: incidence_Day28
Binomial totals: 10
Distribution: Binomial
Link function: Logit
Fitted terms: Constant + BLOCK + Control + Control.Fung + Control.Timing + Control.Fung.Timing

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
Regression	24	542.0	22.582	12.96	<.001
Residual	71	123.7	1.742		
Total	95	665.6	7.007		
Change	-12	-19.0	1.584	0.91	0.542

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ BLOCK	3	3.758	1.253	0.72	0.544
+ Control	1	63.977	63.977	36.73	<.001
+ Control.Fung	6	437.247	72.874	41.84	<.001
+ Control.Timing	2	17.974	8.987	5.16	0.008
+ Control.Fung.Timing	12	19.004	1.584	0.91	0.542
Residual	71	123.673	1.742		
Total	95	665.632	7.007		

Predictions from regression model

Response variate: incidence_Day28

	Timing	Control Prediction	s.e.	-5days Prediction	s.e.
Control	Fung				
Control	Control	0.9917	0.01095	*	*
Fact					
	Amistar	*	*	0.7000	0.09475
	Signum	*	*	0.8250	0.07880
	Octave	*	*	1.0000	0.00029
	Karamate	*	*	0.1250	0.06867

Folicur	*	*	0.8500	0.07410
Headland	*	*	1.0000	0.00029
Switch	*	*	1.0000	0.00029

	Timing	-2days Prediction	s.e.	2days Prediction	s.e.
Fact					
	Amistar	0.4750	0.10306	0.7000	0.09475
	Signum	0.4500	0.10267	0.3000	0.09475
	Octave	1.0000	0.00029	1.0000	0.00029
	Karamate	0.0750	0.05479	0.1750	0.07889
	Folicur	0.7500	0.08963	0.7250	0.09249
	Headland	1.0000	0.00029	1.0000	0.00029
	Switch	0.9750	0.02466	0.9750	0.02466

Analysis of variance (Experiment 1, disease severity, 28 days after inoculation)

Variate: logitseverity28

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK	3	0.24536	0.08179	1.16	0.330
Control	1	13.01131	13.01131	184.92	<.001
Control.Fung	6	19.50405	3.25068	46.20	<.001
Control.Timing	2	1.20603	0.60301	8.57	<.001
Control.Fung.Timing	12	1.72429	0.14369	2.04	0.033
Residual	71	4.99581	0.07036		
Total	95	40.68685			

Grand mean -4.479

BLOCK	1	2	3	4
	-4.459	-4.422	-4.473	-4.560

Control	Control	Fact
	-3.505	-4.618
rep.	12	84

Control	Fung	Control	Amistar	Signum	Octave	Karamate	Folicur
Control		-3.505					
Fact			-5.024	-5.020	-4.265	-5.238	-4.777

Control	Fung	Headland	Switch
Fact		-4.027	-3.974

Control	Timing	Control	-5days	-2days	2days
Control		-3.505			
	rep.	12			
Fact			-4.490	-4.585	-4.778
	rep.		28	28	28

ControlFung	Timing	Control	-5days	-2days	2days	
Control	Control		-3.505			
		rep.	12			
Fact	Amistar			-5.007	-5.115	-4.950
		rep.		4	4	4
	Signum			-4.855	-5.005	-5.199
		rep.		4	4	4
	Octave			-4.037	-4.118	-4.641
		rep.		4	4	4
	Karamate			-5.234	-5.260	-5.221
		rep.		4	4	4
	Folicur			-4.512	-4.972	-4.847
		rep.		4	4	4
	Headland			-3.792	-3.843	-4.447
		rep.		4	4	4
	Switch			-3.994	-3.784	-4.142
		rep.		4	4	4

Standard errors of differences of means

Table	BLOCK	Control	Control Fung	Control Timing	
rep.	24	unequal	12	unequal	
d.f.	71	71	71	71	
s.e.d.				0.1083X	min.rep
	0.0766	0.0819	0.1083	0.0915	max-min
				0.0709	max.rep

Table	Control Fung Timing				
rep.	unequal				
d.f.	71				
s.e.d.	0.1876				min.rep
	0.1531				max-min
	0.1083X				max.rep

(No comparisons in categories where s.e.d. marked with an X)

Least significant differences of means (5% level)

Table	BLOCK	Control	Control Fung	Control Timing	
rep.	24	unequal	12	unequal	
d.f.	71	71	71	71	
l.s.d.				0.2159X	min.rep
	0.1527	0.1632	0.2159	0.1825	max-min
				0.1414	max.rep

Table	Control	
	Fung	
	Timing	
rep.	unequal	
d.f.	71	
l.s.d.	0.3740	min.rep
	0.3054	max-min
	0.2159X	max.rep

Back-transformed means

Fung Timing	Control	Amistar	Signum	Octave	Karamate
Control	2.4178	*	*	*	
-5days	*	0.1646	0.2732	1.2352	0.0303
-2days	*	0.0969	0.1657	1.1015	0.0169
2days	*	0.2034	0.0489	0.4558	0.0372

Fung Timing	Folicur	Headland	Switch
Control	*	*	*
-5days	0.5853	1.7053	1.3084
-2days	0.1885	1.5988	1.7223
2days	0.2791	0.6581	1.0635

Regression analysis (Experiment 2, effect of fungicides on spore germination)

Regression analysis

Response variate:	Total_number_germinated
Binomial totals:	200
Distribution:	Binomial
Link function:	Logit
Fitted terms:	Constant, Treatment

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
Regression	7	1775.8	253.69	8.81	<.001
Residual	16	460.9	28.81		
Total	23	2236.7	97.25		
Change	-7	-1775.8	253.69	8.81	<.001

Dispersion parameter is estimated to be 28.8 from the residual deviance.

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Treatment	7	1775.82	253.69	8.81	<.001
Residual	16	460.91	28.81		
Total	23	2236.72	97.25		

Predictions from regression model

Response variate: Total_number_germinated

	Prediction	s.e.
Treatment		
Control	0.9783	0.03139
Amistar	0.1083	0.06807
Signum	0.1567	0.07963
Octave	0.6450	0.10484
Karamate	0.5267	0.10940
Folicur	0.6283	0.10589
Headland	0.5667	0.10858
Switch	0.8350	0.08127