Project Title:	The potential of the coriander bacterial blight pathogen to infect parsley
Project number:	FV 403
Project leader:	Dr Steven J Roberts
Report:	Final Report, May 2012
Previous report	None
Key staff:	Dr S J Roberts
Location of project:	Plant Health Solutions, Warwick; HDRA, Ryton, Coventry;
Project coordinators:	Mr Tom Davies
Date project commenced:	01 January 2012
Date completion due:	31 May 2012
Keywords:	diseases; bacteria; herbs; coriander; parsley; *Pseudomonas*; *syringae*; coriandricola

DISCLAIMER

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report are based on a series of experiments conducted over a two-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

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

Dr S J Roberts
Director
Plant Health Solutions

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

Report authorised by:

Dr S J Roberts
Director
Plant Health Solutions

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

Table of Contents

GROWER SUMMARY

Headline

UK strains of the coriander bacterial blight pathogen, *Pseudomonas syringae* pv. *coriandricola*, have been confirmed as being able to infect and cause leaf spots on parsley.

Background and objectives

Parsley and coriander are at present the two most important herb crops in the UK, both as field-grown and protected crops. Bacterial blight, caused by *Pseudomonas syringae* pv. *coriandricola* (*Psc*), is the most important disease of coriander. The pathogen is seed-borne and HDC project FV 318 established the importance of and set seed health tolerance standards for seed-borne infection.

A recent scientific paper from the USA (Bull *et al.* 2011) suggested that the seed-borne coriander bacterial blight pathogen (*Psc*) is able to infect not only coriander but also parsley and celery. These results contradict earlier studies done in Germany (Toben et al. 1996) indicating that *Psc* is not a pathogen of parsley or celery. Until now, there has been no reason to doubt these earlier studies and they were used as the basis for information provided in HDC factsheet 16/10, on coriander bacterial blight.

As *Psc* is seed-borne, and coriander and parsley are often grown in close proximity (i.e. in the same field or glasshouse), there would be a significant risk if a pathogen was able to infect both hosts. This potential for cross-infection, if confirmed, could have considerable implications for epidemiology and bacterial disease control. On the other hand, if the US report is correct, it is surprising that there have been no reports of bacterial disease in UK parsley crops, bringing the validity of the US work into question and warranting further verification.

This project aimed to verify US findings using recent and historical UK strains of *Pseudomonas syringae* pv. *coriandricola* and thereby clarify the potential for the seed-borne coriander pathogen to infect parsley.

Summary

Parsley and coriander plants were raised from seed in a glasshouse and inoculated with ten different isolates of *Psc*. These isolates were obtained from coriander leaves, stems and seed in the UK between 1967 and 2011, plus the type strain of the pathogen isolated in Germany in 1990. The majority of the UK isolates (six out of nine), plus the type strain from

Germany, were pathogenic on both coriander and parsley, producing typical bacterial disease symptoms of dark water-soaked lesions on the leaves of both hosts (Figure 1).

Figure 1. Typical disease symptoms on parsley (left) and coriander (right) 16 days after inoculation with *Pseudomonas syringae* pv. *coriandricola*.

This confirms recent results obtained by workers in the USA (Bull *et al.*, 2011), and contradicts earlier work from Germany (Toben & Rudolph, 1996). The results have important implications for herb growers because of the proximity in which the two species are often grown in commercial practice. *Psc* is seed-borne, and HDC project FV 318 showed that infestation of coriander seed lots with *Psc* is relatively common. The difficulties of controlling bacterial diseases together with the potential for cross-infection means that it is now even more important to ensure the health status of coriander seed to prevent introduction of inoculum. Furthermore, since there has never been any testing of parsley seed for the presence of *Psc*, it is not known whether *Psc* can be carried in parsley seed or transmitted from seed to seedling. Parsley seed may therefore also present a risk for coriander crops, so growers / seed companies should consider testing parsley seed for the presence of *Psc*.

The work done in the USA was motivated by the frequent isolation of *Pseudomonas syringae* bacteria from disease outbreaks in parsley in California. It seems curious that despite the prevalence of *Psc* in coriander seed, there have been no reports of bacterial disease in parsley in the UK. One possibility is that this is a result of misdiagnosis: the most common disease in parsley is considered to be a fungal leaf spot caused by *Septoria*

petroselini; it is possible that, in the absence of laboratory diagnosis, bacterial leaf spots on parsley have been assumed to be caused by *Septoria,* when they were in fact caused by *Psc.* This could account for some reports of difficulties in controlling *Septoria* and emphasises the need for laboratory investigation of new or unusual disease symptoms.

There were indications that isolates were less aggressive on parsley than on coriander, and that parsley stems or petioles may be less susceptible to infection than leaves. If proven correct, it is possible that these preliminary observations of differences in tissue susceptibility could lead to differences in the epidemiology of the pathogen in the different hosts. This and possibly other factors that result in relatively reduced 'fitness' of *Psc* on parsley compared to coriander under UK conditions could also provide an alternative explanation of the lack of reports of disease on parsley in the UK.

A few isolates from coriander produced weak or no reactions on parsley. These isolates were also less aggressive on coriander than other isolates. It is possible that this could be indicative of some degree of race specificity, but as these also tended to be older isolates, it is possible that they have lost some pathogenicity during repeated sub-culturing.

Further work would be needed to quantify the relative aggressiveness and 'fitness' of isolates on the two hosts under different conditions and devise and validate seed test methods for the pathogen in parsley.

Financial benefits

Parsley and coriander are the two most important field grown herbs. The project has provided information for determining disease management strategies in these crops and will be used to update HDC factsheet 16/10.

Action points for growers

- Be aware of the potential for cross-infection between parsley and coriander.
- Inspect parsley crops for signs of bacterial disease.
- Do not assume that all leaf spots on parsley are caused by *Septoria petroselini*.
- Ensure that coriander seed has been tested and found free from infestation with *Psc* according to the standards recommended in FV 318.
- Consider having parsley seed tested for *Pseudomonas syringae* pv. *coriandricola* as well as *Septoria petroselini*.

SCIENCE SECTION

Introduction

Background and objectives

Parsley and coriander are at present the two most important herb crops in the UK, both as field-grown and protected crops. Bacterial blight, caused by *Pseudomonas syringae* pv. *coriandricola* (*Psc*), is the most important disease of coriander. The pathogen is seed-borne and a previous HDC project (FV 318) (Green & Roberts, 2010) established the importance of and set seed health tolerance standards for seed-borne infection.

A recent scientific paper from the USA (Bull et al. 2011) suggested that the seed-borne coriander bacterial blight pathogen (*Psc*) is able to infect not only coriander but also parsley and celery. These results contradict earlier studies done in Germany (Toben et al. 1996) indicating that *Psc* is not a pathogen of parsley or celery. Until now, there has been no reason to doubt these earlier studies and they were used as the basis for information provided in the current HDC factsheet on coriander bacterial blight (Roberts & Green, 2010).

As *Psc* is seed-borne, and coriander and parsley are often grown in relatively close proximity (in the same field or glasshouse), there would be a significant risk if a pathogen was able to infect both hosts. This potential for cross-infection, if confirmed, could have considerable implications for epidemiology and bacterial disease control. On the other hand, if the US report is correct, it is surprising that there have been no reports of bacterial disease in UK parsley crops, bringing the validity of the US work into question and warranting further verification.

This short project aims to verify the US findings using recent and historical UK strains of *Pseudomonas syringae* pv. *coriandricola* and thereby clarify the potential for the seed-borne coriander pathogen to infect parsley.

Materials and Methods

Isolates

The isolates used in the study and their origins are listed in Table 1. For long-term storage, isolates were stored in nutrient broth plus 15% glycerol (Feltham *et al.*, 1978); for short-term storage, isolates were maintained on slopes of *Pseudomonas* Agar F (PAF, Difco) medium

at 4°C. Prior to inoculation, isolates were sub-cultured onto plates of PAF and grown for 2 d at 25°C.

Plant raising

Coriander (Hild U804A, previously tested for *Psc* and found to be free) and parsley cv. Gigante d'Italia seeds were sown, 4-5 seeds per cell, in P40 module trays of Fertile Fibre modular seed compost, in a glasshouse. The glasshouse settings were (day/night): minimum 19/15°C with venting at 20/20°C, and with supplementary lighting to provide a minimum of 12 h light. Once plants were sufficiently grown, they were potted on into 7 cm square pots of Fertile Fibre multi-purpose compost.

Table 1. List of isolates of *Pseudomonas syringae* pv. *coriandricola* used in the study, and their origins.

Isolate No.	Synonyms	Year	Part	Country
82B	=NCPPB 3115	1968	leaf	UK
96A	=NCPPB 3117	1967	?	UK
832A	=NCPPB 3116	1974	stem	UK
3126A		1992	leaf	UK
9021		2007	leaf	UK
9039		2008	leaf	UK
9044		2009	seed	UK
9307		2011	seed	UK
9310		2011	seed	UK
9464	=NCPPB 3781	1990	?	Germany

Inoculation

Plants were inoculated at about 6-7 weeks after sowing. Inocula were prepared by suspending a small amount of growth from a 48 h PAF plate in sterile de-ionised water to give a just visibly turbid suspension. The numbers of bacteria in the inocula were estimated by dilution and plating on PAF medium by the drop method of Miles and Misra (1933). Three inoculation methods were used:

1. Infiltration: bacterial suspension was infiltrated into a 5 mm diameter area of ca. six leaflets using a syringe gently pressed against the underside of the leaves.

2. Carborundum/swab: carborundum (silicon carbide) was sprinkled onto the upper-side of ca. six leaflets, and inoculum applied by rubbing the surface with a cotton swab dipped in the bacterial suspension.
3. Stab: the crown of the plant was stabbed with an insect pin charged with bacterial growth from a 48 h PAF plate.

Following inoculation, plants inoculated by methods 1 and 2 were enclosed in polythene bags for 48 h to maintain high humidity; plants inoculated by method 3 were not enclosed in bags. All plants were kept in the shade for 48 h after inoculation.

Plants were monitored at regular intervals following inoculation and the appearance of symptoms recorded.

Re-isolation

Lesions or parts of lesions (2-4 mm^2) were comminuted in drops of sterile de-ionised water on a sterile microscope slide, allowed to stand for a few minutes, then streaked out on plates of PAF medium. Plates were incubated at 25°C for 2-3 d.

Results

The inoculation results are summarised in Table 2, and described in more detail for each method below. Glasshouse temperature averaged around 18°C during the experiment. Isolates were considered to be pathogenic if they produced dark progressive water-soaked lesions by at least one of the three inoculation methods. Nine of the ten isolates were confirmed as pathogenic on coriander. Seven of the nine isolates pathogenic on coriander were also pathogenic on parsley. Isolates appeared to be less aggressive on parsley than on coriander.

Re-isolations from parsley plants inoculated with isolates 9021, 9044, 9307, 9310 all yielded pure cultures that appeared identical to the inoculated isolates.

One isolate (96A) gave only a weak reaction by infiltration on coriander, and no reaction by other methods on either host.

Table 2. Summary of inoculation results for each isolate.

Isolate(s)	Method 1 (infiltration)		Method 2 (swab)		Method 3 (stab)	
	Coriander	Parsley	Coriander	Parsley	Coriander	Parsley
82B	(-)	(-)	(-)	(-)	+, systemic	-
96A	(+)	(-)	-	-	-	-
832A	(+)	(-)	(+)	-	+	-
3126A	+	(+)	+	(+)	+, systemic	+
9021, 9044, 9310	+	+	+	+	+, systemic	-
9039	+	(-)	+	(+)	+, systemic	-
9307	+	(-)	+	+	+, systemic	-
9464	+	(+)	+	+	+, systemic	+
Control	-	-	-	-	-	-

Key to symbols:

Method 1: + = pale dry centre with dark, progressive water-soaked margins, (+) = weak reaction, (-) = pale dry centre only

Method 2: + = dark, progressive water-soaked spots with chlorotic halo, (+) = weak reaction, (-) = pale flecks

Method 3: + = dark water-soaked stem or leaf lesions, systemic = leaf symptoms developing on non-inoculated leaves

Method 1 (Infiltration)

By six days after inoculation, the infiltrated areas of all the inoculated parsley leaves were pale, dry and necrotic; infiltrated areas of coriander leaves also showed a similar response but took slightly longer to develop. By eight days after inoculation, infiltrated areas of coriander leaves were also pale, dry and necrotic. However, most infiltrated coriander leaves were also beginning to show a progressive dark water-soaked margin by eight days, whereas in parsley the development of a water-soaked margin was delayed or sometimes absent.

Method 2 (carborundum/swab)

Six days after inoculation, small dark water-soaked spots, typical of infection with *Psc*, first became apparent on carborundum-inoculated coriander leaves. By eight days after inoculation dark water-soaked spots also began to become apparent on carborundum-inoculated parsley leaves. As lesions progressed the centres tended to dry out and become pale. By 14 to 16 d after inoculation, lesions were clearly visible on both hosts, but had often coalesced to produce large dry necrotic areas (i.e. blight) on coriander.

Method 3 (stab)

By eight days after inoculation, symptoms of water-soaking of the stem or inoculated leaves began to become apparent in coriander, but not parsley. In coriander there was also evidence of systemic spread (i.e. lesions developing on in leaves which had not been inoculated) by 16 d after inoculation. Clear lesions developed in parsley for only two isolates, and there was no sign of systemic spread.

Discussion

The majority of UK isolates of *Psc*, plus the original type strain from Germany, were pathogenic on both coriander and parsley. This verifies the recent findings obtained by workers in the USA (Bull *et al.*, 2011), and contradicts the earlier work from Germany (Toben & Rudolph, 1996). These results have important implications for herb growers, as both parsley and coriander are frequently grown in close proximity, either in the same field or in the same glasshouse. *Psc* is seed-borne, and a previous HDC project, FV 318 (Green & Roberts, 2010) showed that contamination of coriander seed lots with *Psc* is relatively common. The difficulties of controlling bacterial diseases, and the potential for cross-infection therefore means that it is even more important to assure the health status of coriander seed, and prevent introduction of inoculum. Furthermore, since there has never been any testing of parsley seed for the presence of *Psc*, it is not known whether *Psc* can be carried in parsley seed, or transmitted from seeds to seedlings. Parsley seed may therefore present a risk for coriander crops. Growers and seed companies should therefore consider testing parsley seed for the presence of *Psc*.

The work done in the USA was motivated by the frequent isolation of *Pseudomonas syringae* bacteria from disease symptoms/outbreaks in parsley in California. It seems curious that despite the prevalence of *Psc* in coriander seed, there have been no reports of bacterial disease in parsley in the UK. One possibility is that this is a result of misdiagnosis: the most common disease in parsley is considered to be a fungal leaf spot caused by *Septoria petroselini;* it is possible that, in the absence of laboratory diagnosis, bacterial leaf spots on parsley have been assumed to be caused by *Septoria,* when they were in fact caused by *Psc.* This could account for some reports of difficulties in controlling *Septoria* and emphasises the need for laboratory investigation of new/unusual disease symptoms.

Although there is no doubt that *Psc* can infect and cause disease on parsley leaves, the failure of most isolates to give positive reactions by stabbing into the crown of the plant (method 3) suggests that there may be differences in the biology and aetiology of disease on the two hosts. The stab inoculation method is more dependent on the stem or petiole

tissues being susceptible to infection than the other two inoculation methods; it is possible that the relative susceptibility of leaf, petiole and stem tissues differs between the two hosts under the conditions of the experiments. Such differences in susceptibility of different plant parts to infection with particular bacterial plant pathogens is not unknown and has been shown to occur in pea bacterial blight caused by *Pseudomonas syringae* pv. *pisi* (Elvira-Recuenco *et al.*, 2003). If proven correct, it is possible that these preliminary observations of differences in tissue susceptibility could lead to differences in the epidemiology of the pathogen in the different hosts, e.g. reduced susceptibility in the stem in parsley may make seed contamination less likely as a route for disease transmission..

Alternatively there may be other factors that result in relatively reduced 'fitness' of *Psc* on parsley compared to coriander under UK conditions, compared to California, for example ability to survive epiphytically on leaves or in/on the seed, rates of multiplication in the two hosts.

A few isolates from coriander produced weak or no reactions on parsley; these isolates were also less aggressive on coriander than other isolates. It is possible that this could be indicative of some degree of race specificity, but as these also tended to be older isolates, it is possible that they have lost some pathogenicity during repeated sub-culturing.

Further work would be needed to quantify the relative aggressiveness and 'fitness' of isolates on the two hosts, under different conditions and devise and validate seed test methods for the pathogen in parsley.

Conclusions

- UK isolates of *Pseudomonas syringae* pv. *coriandricola* from coriander crops and seed are pathogenic on parsley.

Acknowledgements

The type strain of *Psc* was kindly provided by Fera. Some of the older isolates were isolated by J.D. Taylor at the former National Vegetable Research Station/Horticulture Research International, Wellesbourne, Warwick.

Knowledge and Technology Transfer

The results have been highlighted at the Herb Growers technical meeting and in the HDC weekly e-mail.

References

Bull CT, Clarke CR, Cai R, Vinatzer BA, Jardini TM & Koike ST (2011). Multilocus sequence typing of *Pseudomonas syringae Sensu Lato* Confirms previously described genomospecies and permits rapid identification of *P. syringae* pv. *coriandricola* and *P. syringae* pv. *apii* causing bacterial leaf spot on parsley. *Phytopathology* **101**, 847-858.

Elvira-Recuenco M, Bevan JR & Taylor JD (2003). Differential responses to pea bacterial Blight in Stems, Leaves and Pods Under Glasshouse and Field Conditions. *European Journal of Plant Pathology* **109**, 555-564. http://dx.doi.org/10.1023/A:1024798603610

Feltham RKA, Power AK, Bell PA & Sneath PHA (1978). A simple method for the storage of bacteria at -76°C. *Journal of Applied Bacteriology* **44**, 313-316.

Green K & Roberts SJ (2010). Outdoor herbs: Integrated management of parsley septoria and coriander bacterial blight. Final Report 2007-10, FV 318. pp. 62.

Roberts SJ & Green K (2010) *Coriander bacterial blight. HDC Factsheet 16/10.,* Stoneleigh: HDC.

Miles A.A. & Misra, S.S. (1933). The estimation of the bactericidal power of the blood. *Journal of Hygiene, Cambridge* **38**, 732-749.

Toben H-M & Rudolph K (1996). *Pseudomonas syringae* pv. *coriandricola,* incitant of bacterial umbel blight and seed decay of coriander (*Coriandrum sativum* L.) in Germany. *Journal of Phytopathology* **144**, 169-178.

