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The results and conclusions in this report are based on an investigation 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 P Gladders (for Dr K.R. Green)

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CONTENTS

GROWER SUMMARY

Headline

Pythium and *Fusarium* species are potentially the most important pathogens causing damping-off disease in spinach. Seed stocks that germinated rapidly were less severely affected by damping-off in this project.

Background and expected deliverables

Damping-off was identified as a major problem on UK baby-leaf spinach in late summer 2008. Crops were affected particularly at the cotyledon stage and at canopy closure. In some cases losses were severe, with one grower losing a whole planting of a particular variety. Problems were less severe in 2009 (following a largely dry season) but growers remain concerned that management options are limited. The overall aim of this project was to determine the causal pathogens, provide a clearer understanding of the factors that contribute to outbreaks of spinach damping-off, and to evaluate management practices. The specific objectives are to:

1. Confirm the pathogens most commonly causing damping-off disease on spinach in the UK;
2. Determine the effect of cultivation and environmental factors on the development of damping-off on spinach;
3. Determine the efficacy and persistence of seed treatments and pre-emergence fungicide soil treatments against spinach damping-off.

Summary of the project and main conclusions

Sample collection

Samples were obtained from 11 growers in September 2010, from problem areas that developed following heavy rainfall in August. Losses reached 70-80% in the most severely affected areas. Earlier in the season, few problems were encountered. Damping-off occurred in various crop rotations and despite the use of seed treatments.

Isolations and identification

Pythium and *Fusarium* species were frequently isolated from seedlings with damping-off and are likely to be the main pathogens in the 2010 crops. *Pythium ultimum* and *Pythium* (Hyphal

Swelling group) isolates were shown to be the main pathogens in this project. Growers need to be aware that other pathogens could cause problems as well.

Pathogenicity tests

Fusarium isolates caused leaf rotting in pathogenicity tests. Both *Pythium* and *Fusarium* isolates when added to soil-based compost did not show strong pathogenicity in all experiments. *Pythium* isolates were the main cause of damping-off.

Fusarium spp. and *Mortierella* sp. were common soil fungi recovered from roots and both were found to enhance growth in pathogenicity and seed treatment experiments.

Seed treatments and pre-emergence fungicide sprays

No control of damping-off was achieved with a range of standard and novel seed treatments under field conditions. Fungicide sprays applied just after drilling also had no effect.

Cultivars and seed quality

There were significant differences in the incidence of damping-off between cultivars in inoculated and field experiments. This may be due to differences in the seed lots rather than to cultivar resistance to damping-off.

The results of standard laboratory seed tests for germination, abnormal seedlings, thousand seed weight and time to reach 50% germination were correlated with plant emergence and damping-off in a replicated experiment with naturally infested soil in seed trays. There were promising indications that seed stocks that germinated rapidly were less severely affected by damping-off.

Financial benefits

Experience from 2008 shows that damping-off can cause significant economic loss even in a single planting (grower estimated loss of £42 k at one farm). From this project, growers will have a clearer understanding of the factors that contribute to outbreaks of spinach damping-off, enabling them to reduce risk and improve management practices. The findings may also be of more generic use for management of damping-off on other field vegetable crops.

Action points for growers

- Maintain a record of spinach cultivars that appear susceptible to damping-off, and environmental conditions that are high risk for the disease.
- Ensure cropping areas have good drainage.

SCIENCE SECTION

Introduction

Damping-off was a major problem on UK baby-leaf spinach in later summer 2008. Crops were affected particularly at the cotyledon stage and at canopy closure. The problem was reported independently by at least three major spinach growers. Treatments with Wakil XL (cymoxanil + fludioxonil + metalaxyl-M) and early in-field treatments with fosetyl-aluminium were not effective in controlling the disease. In some cases losses were severe, with one grower losing a whole planting of a particular variety, due to damping-off late in the season. Problems were less severe in 2009 (following a largely dry season) but growers remain concerned that management options are limited.

From seedling samples sent to ADAS in September 2008, damping-off was found to be due to a *Pythium* species. At present, it is not known whether a single species or a range of *Pythium* species was implicated. Damping-off diseases of spinach may be caused by several pathogens. In a 4-year disease survey in southern Sweden, several pathogenic *Pythium* spp. were isolated from spinach roots. Pathogenicity tests showed *P. ultimum* var. *ultimum* to be the most severe spinach pathogen inducing pre- and post-emergence damping-off as well as root rot of older plants. Also *P. heterothallicum* and *P. tracheiphilum* damaged both seedlings and older plants (Larsson, 1994). Other pathogens isolated frequently in the survey included *Aphanomyces cladogamus*, *Phytophthora cryptogea* and *Fusarium oxysporum*. *Rhizoctonia solani* was found only occasionally (Larsson & Olofsson, 1994). No clear relationships were found between pathogen prevalence and disease severity index of surveyed field plants. In Georgia, USA, the pathogens most commonly isolated in association with spinach damping-off were *F. oxysporum, F. solani, Pythium* species, *Rhizoctonia solani* and *Fusarium roseum* (Sumner *et al*., 1976). Further elucidation of the major pathogens responsible for damping-off will enable control measures to be targeted accordingly.

Growers have observed various factors that may be contributing to the occurrence and severity of damping-off, including varietal susceptibility, seed vigour, slow germination following a seed coating, excess or poorly timed irrigation, and adverse weather conditions (low temperatures, high rainfall). The relative contribution of these factors to damping-off disease on spinach has not been ascertained.

The scope for controlling damping-off using in-field fungicide applications is limited since fungicide actives approved for spinach are few at present, and once symptoms of damping-off become apparent within a particular sowing, it is often too late to save the crop through fungicide application. Use of a seed treatment (either a conventional fungicide or biological)

that can provide initial protection of the seedling stage against damping-off, is likely to provide a more practical approach. Conventional seed treatments include Wakil XL and Apron XL (metalaxyl-M), which were both effective in reducing rocket downy mildew for up to 3 weeks after sowing, although there was some phytoxicity following higher dose seed treatments (Gladders, 2008). Alternatives to conventional fungicide seed treatments include an experimental material that is being developed by Germains Technology Group with plans for registration as a spinach seed treatment in North America and Europe. This material has been shown to have activity against a range of spinach pathogens including *Pythium* species (Kinsey, 2009; G. Kinsey, pers. comm). Other potential seed treatments may also emerge from HDC project FV 352: Disease management in organic *Brassica* seed and transplants. An alternative approach will be to evaluate fungicides that could be applied to soil pre-emergence or as a soil drench (e.g. metalaxyl-M as SL567A). From discussion with agro-chemical companies, there may be at least two other actives with potential for testing as soil treatments.

The overall aim of this project is to provide a clearer understanding of the factors that can contribute to outbreaks of spinach damping-off, and to evaluate management practices. The objectives in year 1 were to:

1. Confirm the pathogens most commonly causing damping-off disease on spinach in the UK;

2. Commence studies to determine the effect of cultivation and environmental factors on the development of damping-off on spinach;

3. Commence studies to determine the efficacy and persistence of seed treatments and pre-emergence fungicide soil treatments against spinach damping-off.

Spinach damping-off sample collection in 2010

Introduction

From April 2010, spinach growers were requested as part of this project (through the SPGA and HDC), to provide samples of spinach with typical symptoms of damping-off to ADAS. Samples were obtained in order to confirm the pathogens contributing to the disease, and background information was also obtained on cultural practices and environmental conditions associated with outbreaks of damping-off.

Methods

Growers were requested to send around 10 affected whole plants (leaves and roots together with surrounding soil) in a polythene bag, as well as 10 healthy plants from the same planting (in a separate bag). In addition, the following details for each sample area were requested:

- Grower contact details
- Field reference
- Sowing date
- Variety
- Seed treatment applied
- Approximate area of planting affected
- Field cropping history.

ADAS staff also visited three farms where outbreaks had been reported, to collect samples and associated information.

Samples received by ADAS were examined within two days of receipt for obvious symptoms of damping-off before being used for pathogen isolation.

Results and discussion

Because of relatively dry weather in the spring and summer of 2010, outbreaks of spinach damping-off were not reported until September 2010 following heavy rain in August. Samples were sent or collected from 11 farms where symptoms of damping-off had been observed. A summary of observations on development of damping-off at these sites is given in Table 1. Damping-off affected a range of spinach varieties in autumn 2010 (seven in this study), even though for the majority of varieties, seed had been treated with metalaxyl-M. Growers preferred to use Apron XL rather than Wakil XL, as there had been observations of reduced seedling vigour with the latter treatment. In some crops, areas affected by damping-off were closely associated with areas of the field where there had been water-logging at row-ends (Figure 1). In other crops, symptoms of damping-off were more scattered, extending for bed lengths of approximately 20 m (Figure 2), with small patches of affected plants, surrounded by healthy plants at the time of sampling (Figure 3).

Affected fields did not seem to have a common cropping history. Some crops were second spinach crops for 2010, but others had followed different salad crops (lettuce, coriander). There had been a range of previous crops in 2009 (Table 1). One grower observed that

seedlings in rows at bed edges tended to be less affected (particularly if south-facing) than central rows, perhaps due to better soil drying or drainage. It was also noted that irrigation timing and amount is critical for good establishment, but that irrigation followed by unexpected heavy rainfall could provide conditions conducive for damping-off.

For all samples, affected seedlings were compared in the laboratory with healthy seedlings collected in the same proximity. The symptoms of spinach damping-off observed fitted with descriptions by Koike *et al*. (2007). With pre-emergence damping-off, spinach seed and newly germinated seedlings are attacked and rotted prior to emergence above ground. Symptoms of post-emergence damping-off consist of stunted plants, yellowed lower leaves, general poor growth, wilting and eventual collapse and death of plants. More specifically, cotyledons were either yellow and wilting, or remaining green but with evidence of rot at the base where they were in contact with the rotting lower stem and hypocotyl. Cotyledons of affected plants were approximately half the length of healthy plants, and narrower (Figure 4). True leaves (where emerged) tended to still be green, but stunted and smaller than on healthy plants. Roots on affected plants ranged from almost healthy and creamy white, to complete root death (necrosis and withering). Typically, there was a restriction just below the hypocotyl and rust brown / dark brown root lesions were either longitudinal or girdling the root. There was often sloughing away of the outer cortex, together with withering of the main root and few secondary roots. On sample reference 1A, oospores of a *Pythium* species were visible and abundant under high magnification (x 400). Oospores on this sample had an average diameter of 10.3 µm with a wall thickness of 1.5 µm. Seedlings that had been collected from healthy areas of the crop, with no visible leaf symptoms, occasionally had root symptoms indicating early development of damping-off.

Figure 1. Spinach damping-off following water-logging at row-end, Kent, September 2010.

Figure 2. Spinach damping-off extending along rows, Notts, September 2010.

Figure 3. Spinach plants affected by damping-off (central) compared with health plants.

Table 1. Details of spinach samples collected in autumn 2010 from plantings affected with symptoms of damping-off

Sample no.	Date received	Farm location	Sowing date	Variety	Seed treatment	% planting affected	Disease pattern	Cropping history	Crop notes	General site/grower observations
1A 1H	07.09.10	Kent	08.08.10	Mississipi	Thiram No Force	1%	Yellowing & dead leaves. Patchy emergence at end of a few beds with where ground had lain wet. Most plants affected.	2nd crop of spinach in 2010 2 crops of spinach in 2009	No Force used as thought to Reduce seedling vigour No cover	
2A 2H	07.09.10	West Sussex	24.08.10	Toucan	Thiram Metalaxyl-M Force	n/a	n/a	2nd crop of spinach in 2010 wheat in 2009	SL567A on 30.08.10 Aliette 80WG on 03.09.10	
3A 3H	07.09.10	West Sussex	20.08.10	Kavi	Thiram Metalaxyl-M Force	n/a	n/a	2nd crop of spinach in 2010 wheat in 2009	SL567A on 30.08.10 Aliette 80WG on 30.08.10	
4A 4H	07.09.10	West Sussex	25.08.10	Kavi	Thiram Metalaxyl-M Force	n/a	n/a	Lettuce then spinach in 2010 wheat in 2009	SL567A on 30.08.10	
5A 5H	13.09.10	Notts	31.08.10	Carmel	Conventional seed Untreated	Up to 20%	Patches of few plants affected scattered along beds. Classic damping-off.	1st spinach in 2010 stale seedbed Grass clover in 2009	Organic production No N No herbicides or fungicides	Irrigation applied during dry periods eg at 12 mm at drilling then 7-8 mm 24-48 h later Rows at edge of bed (esp. if south-facing are less affected by damping-off. Better drying / drainage?
6A 6H	13.09.10	Notts	28.08.10	Swan	Thiram Metalaxyl-M Force	20 m stretch of approx 4 beds had 70-80% affected	Large patch of affected plants in a few rows. Neighbouring beds unaffected.	Stale seed bed 2nd crop spinach in 2010 Forage maize in 2009	180 kg N/ha No fungicides	Some beds had damping-off type symptoms plus rotting of cotyledon bases, maybe due to late top dressing. Grower notes that Wakil XL can reduce seedling vigour so used Apron.
7A 7H	13.09.10	Notts	30.08.10	Toucan	Thiram Metalaxyl-M Force	20 m stretch of approx 4 beds had 70-80% affected	Large patch of affected plants in a few rows Neighbouring beds unaffected.	Stale seed bed 2nd crop spinach in 2010 Forage maize in 2009	180 kg N/ha No fungicides	
8A 8H	13.09.10	Notts	03.09.10	Sparrow	Thiram Metalaxyl-M Force	Approx 10% of affected beds	Small patches (approx 30 cm diam) along bed.	Wheat in 2009	180 kg N/ha No fungicides	
9A 9H	13.09.10	Notts	28.08.10	Sparrow	Thiram Metalaxyl-M Force	Approx 10% of affected beds	Small patches (approx 30 cm diam) along bed.	Wheat in 2009	180 kg N/ha No fungicides	
10A 10H	16.09.10	Kent	n/a n/a	n/a n/a	n/a n/a	n/a n/a	n/a n/a	n/a n/a	n/a n/a	
11A 11H	21.09.10	Kent	31.08.10	Induraine	Thiram Metalaxyl-M	1%	Beds at north edge of field drilled on slope facing east	Spinach after coriander in 2010	No further info	

Note: Sample codes ‘A’ were affected with damping-off. Sample codes ‘H’ were healthy but were collected from adjacent to affected areas. n/a data not yet available.

Figure 4. Spinach plants affected by damping-off (left) compared with health plants (right).

Isolate collection and identification

Introduction

Methods for isolation of pathogens from pathogen roots were modified from those of Larsson (1994) and Larsson & Oloffsson (1994).

Methods

Roots from healthy and affected plants from each site were plated onto each of the following media. See Appendix 1 for methods of agar preparation:

1. Potato dextrose agar amended with streptomycin (PDA+S) (general fungal media)
2. P_5ARP (selective media for *Pythium* and *Phytophthora* species)
3. SMA (semi-selective medium for *Aphanomyces* species)
4. SMF (semi-selective medium for *Fusarium* species)

For each sample, roots were removed from ten plants. Roots were surface sterilized by placing in muslin, washing in running tap water for 2 h then drying on filter paper in a laminar flow cabinet. Root sections (2 mm) were plated aseptically (four sections per plate) onto two plates of each agar type, then incubated at 20ºC. The plates were assessed approximately 3 and 7 days after plating for the incidence of different root pathogens (e.g. *Pythium, Phytophthora, Rhizoctonia, Fusarium, Aphanomyces*), and other pathogens and contaminants. Suspect root pathogens were sub-cultured onto PDA+S to get pure cultures. Labelled isolates of suspect *Pythium* and *Phytophthora* species were sent as pure cultures on PDA+S for identification to Dr T Pettitt (Eden Project). Identification of *Pythium* species involved the use of dual cultures on cornmeal agar to check for oospores and pairing with a reference isolate of *Pythium sylvaticum* to induce oospore production. Other species were

initially diagnosed to genus level. After pathogenicity tests were completed, pathogens to be used in further experiments were sent to FERA Plant Clinic, for full diagnosis.

Actively growing isolates were maintained on PDA+S for use in ongoing experiments. All isolates were also be stored on labelled PDA+S slopes in the laboratory fridge

Results and Discussion

The most frequently isolated species were *Pythium* and *Fusarium*, which were isolated from apparently healthy as well as affected seedlings. Possible isolates of *Phytophthora* species were also obtained. There was no *Rhizoctonia* or *Aphanomyces*. Other fungi that were isolated but not sub-cultured further included *Mucor* sp., *Penicillium* sp., *Alternaria* sp., *Cladosporium* sp., and *Stemphylium* sp. Details of fungi isolated consistently and maintained to test as possible causes of damping-off are listed in Table 2.

Table 2. Fungi isolated consistently from spinach root samples from fields with damping-off, September 2010

Site number	Isolate code*	Preliminary identification	Final identification
-	Uninoculated control	-	
1	1A-1 FGP	*Pythium* HS group	*Pythium* HS group
	1A-2 SGP	*Pythium* sp.	Not identified
	1A-1 Fusarium	*Fusarium* sp.	*Fusarium* sp.
	1H-1 FGP	*Pythium ultimum* var. *ultimum*	*Pythium ultimum* var.*ultimum*
	1H-2 FGP	*Pythium* HS group	*Pythium* HS group
2	2A-1 FGP	*Pythium* HS group	*Pythium* HS group
	2H-1 Fusarium A	*Fusarium* sp.	*Fusarium* sp.*
	2H-1 Fusarium B	*Fusarium* sp.	*Fusarium* sp.
3	3A-1 FGP	*Pythium ultimum* var. *ultimum*	*Pythium ultimum* var.*ultimum*
	3A-1 Fusarium	*Fusarium* sp.	*Fusarium* sp.
	3H-1 Fusarium A	*Fusarium* sp.	*Fusarium* sp.
	3H-1 Fusarium B	*Fusarium* sp.	*Fusarium* sp.
	3H-1 Phytophthora	*Phytophthora* sp.	*Pythium* HS group + *Mortierella* sp.
4	4A-1 Fusarium	*Fusarium* sp.	*Fusarium* sp.*
	4H-1 FGP	*Pythium ultimum* var. *ultimum*	*Pythium ultimum* var.*ultimum*
	4H-1 SGP	*Pythium* sp.	Not identified
	4H-1 Fusarium?	*Fusarium* sp.	*Fusarium* sp.
5	5A-1 SGP	*Pythium* sp.	*Pythium* HS group
	5H-1 Phytophthora	*Phytophthora* sp.	*Mortierella* sp.
	5H-1 Fusarium	*Fusarium* sp.	*Fusarium* sp.
6	6A-1 Fusarium	*Fusarium* sp.	*Fusarium* sp.
	6H-2 Phytophthora	*Phytophthora* sp.	*Mortierella sp.*
7	7H-1 FGP	*Pythium* sp.	*Pythium ultimum* var.*ultimum*
	7H-1 Phytophthora	*Phytophthora* sp.	Mortierella sp.
8	8A-1 FGP	*Pythium* sp.	*Pythium* HS group
	8H-1 Fusarium	*Fusarium* sp.	*Fusarium* sp.*
9	9A-1 Fusarium	*Fusarium* sp.	*Fusarium* sp.*
	9H-1 Fusarium	*Fusarium* sp.	*Fusarium* sp.*
10	10A-1 FGP	*Pythium* sp.	Not identified
	10H-1 FGP	*Pythium* sp.	*Pythium* HS group
11	11A-1 Fusarium A	*Fusarium* sp.	*Fusarium* sp.
	11A-1 Fusarium B	*Fusarium* sp.	*Fusarium* sp.
	11H-1 Fusarium	*Fusarium* sp.	*Fusarium* sp.

*'A' denotes isolates from field areas affected by damping-off; 'H' denotes isolates from visibly healthy plants adjacent to affected areas. * indicates Pythium Hyphal Swellings group also identified but not used in pathogenicity tests.

FGP – fast growing Pythium; SGP – slow growing Pythium

The identification of *Pythium* species was difficult, particularly as this was not assisted by the dual culture techniques*. Pythium ultimum* var. *ultimum* and *Pythium* species assigned to the Hyphal Swellings group were identified at several of the sites. There could be several different pathogens present in any one crop. A few isolates were not identified where subcultures were not successful and others proved to be mixed cultures where *Mortierella* species were present. *Mortierella* species occur commonly in soil and are early colonists of damaged tissues. There colony morphology is similar to that of some *Pythium* species. There were no confirmed isolates of *Phytophthora* species that were initially thought to be present in initial isolations (Table 2). However, *Phytophthora* species may be affecting spinach seedlings in some crops.

Whilst *Pythium* and *Fusarium* species predominated in these samples, there is a range of other fungal pathogens that could affect spinach crops. These may vary from field to field and in relation to environmental conditions.

Pathogenicity tests

Introduction

The objectives of this part of the study were:

- To compare the pathogenicity of isolates that were obtained consistently from spinach roots (autumn 2010), on spinach using different techniques.
- To select isolates for use in further pot studies that required artificial inoculation of spinach seedling roots.

Methods for experiments 2 and 3 were modified from Larsson & Olofsson (1994).

Methods

In all three experiments, the pathogenicity of 33 fungal isolates (listed in Table 2) on spinach were compared, together with an uninoculated control. In Experiment 1, isolates were tested on spinach leaves. In Experiment 2, fungal inoculum was incorporated into growing media to test isolate effects on germinating spinach seedlings. In Experiment 3, inoculum was placed in a layer within growing media below spinach seeds, to test isolate effects on developing roots.

Experiment 1 (inoculation of detached spinach leaves and petioles)

For each isolate and the uninoculated control, there were four replicate leaves laid out in randomized design.

Spinach leaves were obtained from purchased bags of baby leaf spinach. Only leaves that were free from symptoms of disease or decay, and with petioles attached were used. For each leaf, both surfaces and the petiole were wiped gently with a paper tissue moistened with ethanol. One leaf was placed per Petri dish, and a slit (0.5-1 cm in length) was cut in each petiole using a sterile scalpel. The Petri dishes were labelled with isolate code and replicate number.

For each isolate, an actively growing culture on PDA+S was used. For the uninoculated control treatment, a fresh plate of PDA+S was used. In the laminar flow cabinet, 4 x 5 mm plugs were cut using a cork borer in the clean PDA+S plate, and towards the leading edge of each culture. The cork borer was sterilized in ethanol and flamed thoroughly before and between isolates. Four plugs of clean PDA+S were removed and inserted into petiole slits for four leaves (uninoculated control). Similarly, the mycelial plugs for each isolate were removed and inserted into the spinach petiole slits. The Petri dishes containing the inoculated leaves were sealed with parafilm and incubated at 20^{o}C. At 3 and 8 days after inoculation, the petioles and leaves were assessed for lesion development.

Experiment 2 (inoculation of germinating seedlings using inoculum incorporated through soil)

For each isolate and the uninoculated control, there were 3 replicate pots each sown with 10 spinach seeds, and laid out in a randomized complete block design. Statistical analysis was by GLM in Genstat for incidence of emergence, and by ANOVA in Genstat for severity of root rots. A total of 102 plant pots (9 cm diameter) were half-filled with John Innes no. 2 compost. For each isolate, a sterile scalpel was used to cut actively growing cultures from three plates into 0.5 cm^2 pieces, while clean plates of PDA+S cut into pieces were used for the uninoculated control. The pieces of fungal culture (or PDA+S) were incorporated evenly into compost and this infested compost media was used to fill up three pots per isolate.

20 g spinach seed (cv. Swan, untreated) was rinsed in 1% sodium hypochlorite solution for 30 seconds followed by two 1 minute washes in distilled water, then air-dried on filter paper in a laminar flow cabinet. 10 seeds were sown per pot at approximately 1 cm depth in the infested compost. The pots were placed on trays in a randomized complete block design with three replicates per isolate. The pots were maintained at 18^{o}C, with 12 h day, 12 h night, with regular watering to maintain moist but not waterlogged compost.

Percentage emergence of healthy and stunted seedlings was recorded after 7 and 14 days.

After 14 days, the seedlings were uprooted from each pot and laid on a paper towel. Seedlings were scored according to the following index (modified from Larsson & Gerhardson, 1990):

Plant reaction observed:	**Disease severity index**
No visible symptoms	0
Light browning of less than 5 mm of a single root	5
About 10% of the root system discoloured and affected	10
About 25% of the root system discoloured and affected	25
The whole root system discoloured and affected but no symptoms on hypocotyl or leaves.	50
The whole root system and hypocotyl discoloured and affected but no symptoms on the leaves	75
Plants were dead, or the whole root system, and the hypocotyl was discoloured and affected; leaves were wilted, stunted or yellowing.	100

For at least two seedlings per isolate, sections of affected root pieces were plated onto PDA+S after they had been rinsed under running tap water for about 2 hours, then dried on filter paper in a laminar flow cabinet. Plates were checked after 3 and 7 days to determine whether re-isolations were the same as the original isolates.

Experiment 3 (inoculation of developing roots, using a layer of inoculum in soil)

For each isolate and the uninoculated control, there were 3 replicate pots each sown with 10 spinach seeds, and laid out in a randomized complete block design. Statistical analysis was by GLM in Genstat for incidence of emergence, and by ANOVA in Genstat for severity of root rots.

102 plant pots (9 cm diameter) were 2/3 filled with John Innes no. 2 compost. For each isolate, a sterile scalpel was used to cut out whole cultures from three plates, while clean plates of PDA+S were used for the uninoculated control. The pieces of fungal culture (or PDA+S) were incorporated evenly into compost and this infested compost media was used to fill up three pots per isolate. The fungal cultures (or PDA+S) were laid evenly across the compost surface of three pots before adding another 2 cm depth of compost.

20 g spinach seed (cv. Swan, untreated) was rinsed in 1% sodium hypochlorite solution for 30 seconds followed by two 1 minute washes in distilled water, then air-dried on filter paper in a laminar flow cabinet. 10 seeds were sown per pot, then covered with approx 1 cm depth compost.

Experimental design, maintenance and assessments were as described for Experiment 2.

Results and Discussion

Experiment 1

There was considerable variation in the pathogenicity of the fungal isolates on spinach leaves (Table 3). None of the *Pythium* or suspect *Phytophthora* isolates (subsequently identified as *Mortierella* species) were highly pathogenic on leaves; inoculation with these isolates resulted in slight mycelial growth across the leaf, and occasionally slight lesion development. Given that these organisms were isolated from roots, limited pathogenicity on leaves is unsurprising. In contrast, several of the *Fusarium* isolates were highly pathogenic on leaves, with rapid mycelial growth across the leaves followed by lesion development.

Experiment 2

There was considerable variation in the emergence of spinach seedlings, with significant increases in emergence after 14 days with some isolates (Table 4). The untreated control showed very poor emergence suggesting that the test conditions had adversely affected this seed stock. It is possible that fungi in the compost, perhaps affected by the sterile agar medium, affected emergence. Only two fungal isolates gave significantly better emergence than the untreated and none showed a greater percentage of stunted seedlings. A number of isolates showed very low emergence (0-3%), but this was associated with different fungal species. This experiment did not clearly demonstrate that the isolates were strongly pathogenic from soil inoculum at seedling emergence. This may well be due to the test conditions and techniques was modified in Year 2.

Experiment 3

There were no significant differences between isolates and the untreated control in emergence or stunting of seedlings in this experiment. The *Pythium* isolate 2A-1 and 7H-1 (now thought to be *Mortierella* sp.) both prevented any seedling emergence as they had in Experiment 2. Such trends suggest they may be potential pathogens. Numerous isolates appeared to give improved emergence though the differences were not significant (Table 5). The technique appeared to allow greater earlier emergence than in Experiment 2, but stunting was recorded more consistently across the isolates.

Table 3. Pathogenicity of fungal isolates (*ex* spinach roots) on spinach leaves (Expt. 1)

Isolate code	Isolate identification	Mean % leaf area affected*	Standard deviation	Presence of leaf necrosis (+) **
Uninoculated control	-	0.0	0.0	-
1A-1 FGP	*Pythium* HS group	3.3	4.6	-
1A-2 SGP	Not identified	5.3	6.9	+
1A-1 Fusarium	*Fusarium* sp.	53.8	18.9	+
1H-1 FGP	*Pythium ultimum* var.*ultimum*	0.3	0.5	-
1H-2 FGP	*Pythium* HS group	0.5	1.0	-
2A-1 FGP	*Pythium* HS group	88.0	12.4	+
2H-1 Fusarium A	*Fusarium* sp.	68.8	2.5	+
2H-1 Fusarium B	*Fusarium* sp.	26.3	8.5	+
3A-1 FGP	*Pythium ultimum* var.*ultimum*	4.3	7.2	-
3A-1 Fusarium	*Fusarium* sp.	75.0	7.1	+
3H-1 Fusarium A	*Fusarium* sp.	8.5	7.5	+
3H-1 Fusarium B	*Fusarium* sp.	82.5	11.9	+
3H-1 Phytophthora	*Pythium* HS group + *Mortierella* sp.	0.5	1.0	-
4A-1 Fusarium	*Fusarium* sp.	98.5	1.7	+
4H-1 FGP	*Pythium ultimum* var.*ultimum*	0.0	0.0	-
4H-1 SGP	Not identified	0.0	0.0	-
4H-1 Fusarium?	*Fusarium* sp.	71.3	17.5	+
5A-1 SGP	*Pythium* HS group	1.3	1.5	-
5H-1 Phytophthora	*Mortierella* sp.	0.0	0.0	-
5H-1 Fusarium	*Fusarium* sp.	85.8	18.4	+
6A-1 Fusarium	*Fusarium* sp.	6.8	8.9	-
6H-2 Phytophthora	*Mortierella sp.*	0.0	0.0	-
7H-1 FGP	*Pythium ultimum* var.*ultimum*	0.5	0.6	-
7H-1 Phytophthora	Mortierella sp.	0.0	0.0	-
8A-1 FGP	*Pythium* HS group	1.0	1.4	-
8H-1 Fusarium	*Fusarium* sp.	48.8	17.5	+
9A-1 Fusarium	*Fusarium* sp.	98.3	2.4	+
9H-1 Fusarium	*Fusarium* sp.	91.3	10.3	+
10A-1 FGP	Not identified	0.0	0.0	-
10H-1 FGP	*Pythium* HS group	2.3	2.2	-
11A-1 Fusarium A	*Fusarium* sp.	91.5	14.4	+
11A-1 Fusarium B	*Fusarium* sp.	3.3	1.3	-
11H-1 Fusarium	*Fusarium* sp.	11.0	6.2	+

* Mean of four leaves.

** Some leaves had mycelial development only.

Table 4. Pathogenicity of fungal isolates (*ex* spinach roots) as soil inoculum on emergence of spinach seedlings (Expt. 2)

Isolate code	Isolate identification	% emergence after 7 days	% emergence after 14 days	% stunted 7 days	% stunted 14 days
Uninoculated control	-	0.0	10.0	0.0	50.0
1A-1 FGP	*Pythium* HS group	6.7	13.3	0.0	0.0
1A-2 SGP	Not identified	3.3	20.0	0.0	22.3
1A-1 Fusarium	*Fusarium* sp.	0.0	10.0	0.0	33.3
1H-1 FGP	*Pythium ultimum* var.*ultimum*	6.7	23.3	0.0	22.3
1H-2 FGP	*Pythium* HS group	30.0	53.3	0.0	30.7
2A-1 FGP	*Pythium* HS group	0.0	0.0	0.0	0.0
2H-1 Fusarium A	*Fusarium* sp.	16.7	33.3	0.0	4.7
2H-1 Fusarium B	*Fusarium* sp.	0.0	10.0	0.0	0.0
3A-1 FGP	*Pythium ultimum* var.*ultimum*	6.7	23.3	0.0	30.0
3A-1 Fusarium	*Fusarium* sp.	0.0	16.7	0.0	0.0
3H-1 Fusarium A	*Fusarium* sp.	3.3	10.0	0.0	33.3
3H-1 Fusarium B	*Fusarium* sp.	0.0	3.3	0.0	0.0
3H-1 Phytophthora	*Pythium* HS group + *Mortierella* sp.	0.0	13.3	0.0	0.0
4A-1 Fusarium	*Fusarium* sp.	0.0	20.0	0.0	16.7
4H-1 FGP	*Pythium ultimum* var.*ultimum*	13.3	33.3	0.0	16.7
4H-1 SGP	Not identified	3.3	6.7	0.0	0.0
4H-1 Fusarium?	*Fusarium* sp.	0.0	13.3	0.0	0.0
5A-1 SGP	*Pythium* HS group	10.0	33.3	0.0	11.0
5H-1 Phytophthora	*Mortierella* sp.	3.3	6.7	0.0	16.7
5H-1 Fusarium	*Fusarium* sp.	10.0	36.7	0.0	0.0
6A-1 Fusarium	*Fusarium* sp.	0.0	30.0	0.0	8.3
6H-2 Phytophthora	*Mortierella sp.*	0.0	20.0	0.0	16.7
7H-1 FGP	*Pythium ultimum* var.*ultimum*	3.3	13.3	0.0	16.7
7H-1 Phytophthora	Mortierella sp.	0.0	0.0	0.0	0.0
8A-1 FGP	*Pythium* HS group	6.7	10.0	0.0	11.0
8H-1 Fusarium	*Fusarium* sp.	0.0	3.3	0.0	33.3
9A-1 Fusarium	*Fusarium* sp.	0.0	20.0	0.0	0.0
9H-1 Fusarium	*Fusarium* sp.	0.0	10.0	0.0	0.0
10A-1 FGP	Not identified	0.0	3.3	0.0	0.0
10H-1 FGP	*Pythium* HS group	3.3	16.7	0.0	33.3
11A-1 Fusarium A	*Fusarium* sp.	3.3	20.0	0.0	27.7
11A-1 Fusarium B	*Fusarium* sp.	0.0	10.0	0.0	33.3
11H-1 Fusarium	*Fusarium* sp.	0.0	3.3	0.0	0.0
	SED (61 df)	7.09	12.37	-	24.11
	P	0.056 (ns)	0.016	-	0.845 (ns)
	LSD	14.15	24.70	-	48.14

Table 5. Pathogenicity of fungal isolates (*ex* spinach roots) as soil inoculum on roots of spinach seedlings (Expt. 3)

Isolate code	Isolate identification	% emergence after 7 days	% emergence after 14 days	% stunted 7 days	% stunted 14 days
Uninoculated control	-	13.3	33.3	0.0	24.3
1A-1 FGP	*Pythium* HS group	3.3	50.0	0.0	33.3
1A-2 SGP	Not identified	13.3	40.0	11.0	24.3
1A-1 Fusarium	*Fusarium* sp.	23.3	53.3	0.0	23.0
1H-1 FGP	*Pythium ultimum* var.*ultimum*	0.0	30.0	0.0	43.0
1H-2 FGP	*Pythium* HS group	3.3	43.3	0.0	50.0
2A-1 FGP	*Pythium* HS group	3.3	3.3	0.0	0.0
2H-1 Fusarium A	*Fusarium* sp.	10.0	37.3	0.0	27.7
2H-1 Fusarium B	*Fusarium* sp.	16.7	63.3	33.3	26.7
3A-1 FGP	*Pythium ultimum* var.*ultimum*	6.7	63.3	0.0	22.7
3A-1 Fusarium	*Fusarium* sp.	16.7	80.0	0.0	4.0
3H-1 Fusarium A	*Fusarium* sp.	13.3	56.7	0.0	37.3
3H-1 Fusarium B	*Fusarium* sp.	20.0	56.7	8.3	19.5
3H-1 Phytophthora	*Pythium* HS group + *Mortierella* sp.	10.0	60.0	0.0	26.3
4A-1 Fusarium	*Fusarium* sp.	10.0	30.0	0.0	36.0
4H-1 FGP	*Pythium ultimum* var.*ultimum*	6.7	40.0	0.0	28.0
4H-1 SGP	Not identified	6.7	43.3	0.0	12.3
4H-1 Fusarium?	*Fusarium* sp.	10.0	60.0	0.0	16.7
5A-1 SGP	*Pythium* HS group	0.0	63.3	0.0	21.3
5H-1 Phytophthora	*Mortierella* sp.	6.7	43.3	0.0	27.7
5H-1 Fusarium	*Fusarium* sp.	6.7	66.7	0.0	27.0
6A-1 Fusarium	*Fusarium* sp.	6.7	46.7	0.0	15.7
6H-2 Phytophthora	*Mortierella sp.*	13.3	66.7	0.0	31.7
7H-1 FGP	*Pythium ultimum* var.*ultimum*	13.3	66.7	0.0	24.3
7H-1 Phytophthora	Mortierella sp.	6.7	36.7	0.0	20.0
8A-1 FGP	*Pythium* HS group	6.7	40.0	0.0	39.0
8H-1 Fusarium	*Fusarium* sp.	10.0	56.7	0.0	29.5
9A-1 Fusarium	*Fusarium* sp.	16.7	70.0	0.0	47.3
9H-1 Fusarium	*Fusarium* sp.	6.7	66.7	0.0	14.7
10A-1 FGP	Not identified	20.0	56.7	0.0	33.3
10H-1 FGP	*Pythium* HS group	16.7	53.3	0.0	20.3
11A-1 Fusarium A	*Fusarium* sp.	0.0	36.7	0.0	30.0
11A-1 Fusarium B	*Fusarium* sp.	6.7	46.7	0.0	36.3
11H-1 Fusarium	*Fusarium* sp.	3.3	13.3	0.0	66.7
	SED (61 df)	9.71	19.24	8.74	21.25
	P	0.795 (ns)	0.097 (ns)	0.529 (ns)	0.888 (ns)
	LSD	19.39	38.41	17.45	42.50

Control of damping-off under field conditions

Introduction

As spinach crops are often harvested within four weeks of sowing, seed treatments are of particular interest as an efficient way of using crop protection treatments. Damping-off occurs very soon after sowing and treatments therefore need to be able to provide good protection from sowing onwards. The treatments used in these experiments were those with label recommendations and novel treatments identified by manufacturers as potential treatments for use against damping-off. Thiram was used a standard that could be used to relate efficacy to previous work. In addition to seed treatments, pre-emergence fungicide sprays were also evaluated.

The objectives of the study were:

1. To evaluate new fungicides and biological treatments with potential to control damping-off diseases, particularly *Pythium* species, in soil.
2. To determine if there are differences in cultivar susceptibility to damping-off diseases.
3. To establish if treatments affect growth and vigour of spinach seedlings.

Methods

Experiment 4: Control of damping-off with seed treatments, 2011

There were seven seed treatments on both cvs. Carmel and Stanton and a single novel seed treatment on cv. Stanton only (as product supplies were limited). These 15 treatments were randomised within each of four replicate blocks (total 60 plots) (Table 6).

The site was on a farm in Nottinghamshire with a history of spinach damping-off problems. The soil was a sandy loam pH 7.3 (P index 3, K index 2- and Mg Index 2; 2.0% organic matter). The trial was drilled on 7 September 2011 after beds had been prepared and levelled, using farm equipment (Air drill) that was restricted to 4 coulters (instead of the full 9 coulters) and not using the outer coulter at the bed edge. A 5 m plot length was used so that 1 m at each end of the plot could be discounted from the plot assessments. A soil sample was taken at drilling to determine pH, nutrients and organic matter.

Assessments were made at intervals to determine effects of treatments on crop emergence, damping-off diseases and plant vigour. These were done on the crop established from the two central coulters at early emergence (9 days after sowing, 16 September), 21 and 29 September and 20 October. This was done using three small quadrats (maximum 0.25m^2) per plot and counting the number of healthy seedlings and the number with

damping-off (usually affected seedlings were wilting or had collapsed). Data were then used to calculate plant populations per m^2 and % damping-off. Plant vigour was scored in each plot using a 0-10 scale where 5 is the average vigour.

Table 6. Seed treatments and cultivars used for control of damping-off, Notts 2011 (Expt. 4)

	Seed treatment	Cultivar	Rate of product
1	Untreated	Stanton	Untreated
2	Untreated	Carmel	-
3	Thiram	Stanton	3.6 g/kg seed (75% thiram)
4	Thiram	Carmel	3.6 g/kg seed (75% thiram)
5	Apron XL	Stanton	
6	Apron XL	Carmel	
7	Wakil XL	Stanton	
8	Wakil XL	Carmel	
9	Film coated only	Stanton	-
10	Film coated only	Carmel	-
11	HDC F57	Stanton	
12	HDC F57	Carmel	
13	HDC F58	Stanton	
14	HDC F58	Carmel	
15	HDC F59	Stanton	

Experiment 5: Control of damping-off with cultivars and fungicide spray treatments, 2011

This experiment was sown alongside Experiment 4 on the same day. A factorial design with five cultivars and three fungicide treatments was used, with the three fungicide treatments randomized within each cultivars plot. These 15 treatments had four-fold replication (total 60 plots) (Table 7).

The trial was drilled on 7 September 2011 after beds had been prepared and levelled using farm equipment (Air drill) that was restricted to 4 coulters (instead of the full 9 coulters) and not using the outer coulter at the bed edge. A 15 m plot length was used for each cultivars so that each fungicide treatment had a 5 m plot length.

Assessments were made at intervals to determine effects of treatments on crop emergence, damping-off diseases and plant vigour. These were done on the crop established from the two central coulters at early emergence (9 days after sowing, 16 September), 21 and 29 September and 20 October. This was done using three small quadrats (maximum 0.25 m^2) per plot and counting the number of healthy seedlings and the number with damping-off (usually affected seedlings were wilting or had collapsed). Data were then used to calculate

plant populations per m^2 and % damping-off. Plant vigour was scored in each plot using a 0-10 scale where 5 is the average vigour.

Pre-emergence fungicides will be applied almost immediately after drilling using a CO_2 pressurised Oxford precision sprayer with 110-03 nozzles as a medium-coarse spray at 2 bar pressure in 500 L water/ha.

Table 7. Varieties and pre-emergence fungicides, Notts 2011 (Expt. 5).

	Cultivar	Rate of product
Factor 1 Cultivar		
1	Stanton	
2	Carmel	
3	Silverwhale	
4	Toucan	
5	Pigeon	
Factor 2 Fungicide		
1	Untreated	None
2	Previcur Energy	2.5 kg/ha
3	SL567A	0.12 l/ha

Results

Experiment 4: Control of damping-off with seed treatments, 2011

Damping-off caused by *Pythium* occurred in all treatments and there were more problems in Carmel than Stanton (P=0.025) which averaged 6.0% and 4.6% respectively (Table 8). There were initial suggestions of seed treatment activity on 16 September 2011 when no damping-off was found in thiram or the coded treatment HDC F59 (Table 8). Damping-off incidence differed significantly between assessments (P<0.001) with the highest incidence (mean 9.3% of plants affected) on 21 September. As damping-off affected and killed seedlings at emergence, the small plants quickly shriveled and disappeared in a few days. The numbers of plants with damping-off therefore varies between assessments reflecting progress of severe disease and loss of dead plants. There was a variety x assessment date interaction (P=0.038), notably where damping-off continued to increase until 29 September in the Apron XL treatment. No treatments gave control of damping-off, but some treatments such as Film coating and some of the coded treatments resulted in higher damping-off on Carmel than Stanton. Final plant counts showed variation in populations between treatments, but no cultivars or seed treatment effects were significant.

Table 8. Effects of seed treatments and cultivars on damping-off and plant counts, Notts 2011 (Expt. 4)

	Seed treatment	Cultivar	Rate of product	% damping-off 16 Sept	% damping-off 21 Sept	% damping-off 29 Sept	Total plants/m^2 20 0ct
1	Untreated	Stanton	Untreated	0.8	5.9	2.4	484.0
2	Untreated	Carmel	-	3.1	11.0	4.0	492.0
3	Thiram	Stanton	3.6 g/kg seed (75% thiram)	0.0	5.3	3.7	472.0
4	Thiram	Carmel	3.6 g/kg seed (75% thiram)	0.7	10.1	5.5	440.0
5	Apron XL	Stanton		1.3	6.8	2.0	488.0
6	Apron XL	Carmel		2.8	6.6	7.5	508.0
7	Wakil XL	Stanton		3.1	10.8	7.0	476.0
8	Wakil XL	Carmel		3.9	9.0	5.4	304.0
9	Film coated only	Stanton	-	1.1	9.2	3.4	444.0
10	Film coated only	Carmel	-	4.8	12.0	2.9	444.0
11	HDC F57	Stanton		1.1	7.7	4.6	500.0
12	HDC F57	Carmel		1. 5	10.1	4.6	432.0
13	HDC F58	Stanton		0.3	13.5	4.0	420.0
14	HDC F58	Carmel		1.8	13.9	3.8	484.0
15	HDC F59	Stanton		0.0	8.2	3.9	512.0
			Mean	1.8	9.3	4.3	460.0
	Variety means	Stanton					472.0
		Carmel					442.0
		Overall	F test	0.033	NS 0.125	NS 0.209	NS 0.249
		Variety					
		Variety x ST					
			SED	1.436	2.972	1.855	64.3
			LSD	2.899	5.998	3.743	129.6

Experiment 5: Control of damping-off with cultivars and fungicide spray treatments, 2011

Differences between cultivars in the incidence of damping-off (caused by *Pythium)* were significant on 29 September and when averaged over the three assessment dates (Table 9). However, there were no significant effects from pre-emergence sprays. The incidence of damping-off differed between assessment dates and was greatest two weeks after sowing. Stanton had the lowest damping-off score at all three assessments (Table 9). Silverwhale was the cultivar most severely affected by damping-off and this was significantly greater than in all the other cultivars.

Table 9. Damping-off in cultivar x fungicide spray treatments, Notts 2011 (Expt. 5)

	Cultivar	Rate of product	% damping-off 16 Sept	% damping-off 21 Sept	% damping-off 29 Sept	Mean % damping off
Factor 1 Cultivar						
1	Stanton		3.2	9.1	3.9	5.4
2	Carmel		6.1	12.4	5.2	7.9
3	Silverwhale		7.0	19.8	9.1	12.0
4	Toucan		3.9	12.5	5.3	7.3
5	Pigeon		3.3	14.3	7.5	8.4
	Mean		*4.7*	*13.6*	*6.2*	*8.2*
Factor 2 Fungicide						
1	Untreated	None	4.7	14.1	5.7	8.2
2	Previcur Energy	2.5 kg/ha	3.9	14.5	6.2	8.2
3	SL567A	0.12 l/ha	5.4	12.2	6.7	8.1
	Mean		*4.7*	*13.6*	*6.2*	*8.2*
		F test Cultivar	NS 0.217	NS 0.080	0.045	<0.001
		F test Fungicide	NS 0.314	NS 0.352	NS 0.525	NS 0.992
		F test cv x fung	NS 0.628	NS 0.687	NS 0.563	NS 0.772
		F test date assessed				<0.001
		LSD Cultivar	4.16	7.388	3.523	2.38
		LSD Fungicide	1.968	3.385	1.65	1.844
		LSD cv x fung	5.29	9.271	4.46	4.123
		LSD date assessed				1.749

Table 10. Damping-off in cultivar x fungicide spray treatments, Notts 2011 (Expt. 5)

	Cultivar	Rate of product	Total plants/m^2 20 Oct
Factor 1 Cultivar			
1	Stanton		458.7
2	Carmel		370.7
3	Silverwhale		430.7
4	Toucan		430.7
5	Pigeon		445.3
	Mean		*427.2*
Factor 2 Fungicide			
1	Untreated	None	434.4
2	Previcur Energy	2.5 kg/ha	426.4
3	SL567A	0.12 l/ha	420.8
	Mean		*427.2*
		F test Cultivar	0.046
		F test Fungicide	NS 0.834
		F Test cv x fung	NS 0.827
		LSD Cultivar	59.05
		LSD Fungicide	45.74
		LSD cv x fung	102.28

There were significant differences in final plant stands on 20 October (P=0.046) with Carmel having fewer plants than all the other varieties. There was no effect of fungicide treatment on plant population (Table 10). Carmel also had a lower plant population than Stanton in Expt. 4 (see Table 8), though differences were not significant in that experiment.

Pathogenicity testing and control with novel seed treatments

Methods

Experiment 6: Pathogenicity of fungal isolates on different cultivars and effect of seed treatments

This pot experiment was sown on 1 November 2011 in John Innes seeding compost under cold glasshouse conditions at ADAS Rosemaund, near Hereford. Six representative fungal isolates of *Pythium*, *Fusarium* and *Mortierella* (Table 2) were tested on three cultivars (Squirrel, Swan and Toucan), each with two novel seed treatments (see Table 11). A fully randomised block design was used with 42 treatments including untreated controls for each pathogen and replicated 4 times. A compost sample was taken for standard nutrient analyses.

All fungal isolates were maintained on potato dextrose agar. Inoculum was produced by growing the test isolates on a sterilized millet substrate in distilled water using 250 ml flasks. The flasks/bags were shaken regularly over a four week period to ensure even colonization of the substrate.

The inoculum for each pathogen was thoroughly mixed into commercial seedling compost at a rate of 1% w/w and added to one litre size half pots (14 cm diameter). Twenty seeds were laid evenly spaced across the surface of the compost and pushed in gently to a depth of 1cm. Each pot was placed on an individual saucer and watered until water emerged from the base of the pot. Subsequent watering was done by filling the saucer.

The total number of emerged seedlings and those with damping-off symptoms were counted every few days from first emergence. Plant vigour was recorded on a 0-10 scale. On 9 December, the severity of root symptoms on seedlings was assessed using the 0-100 index after they had been removed from the compost and washed.

Experiment: Pathogenicity of fungal isolates on different cultivars

This pot experiment was done using the same methods as Experiment 6 to determine the effects of the test fungal isolates on untreated seed of the same three varieties (Squirrel, Swan and Toucan) sown on 20 February 2012 in John Innes seeding compost under cold glasshouse conditions at ADAS Rosemaund, near Hereford. Six representative fungal isolates were tested on three varieties (Squirrel, Swan and Toucan). A fully randomised block design was used with 21 treatments including untreated controls and replicated 4 times.

The fungal isolates and inoculation methods were as described for Experiment 6. Twenty seeds were laid evenly spaced across the surface of the compost and pushed in gently to a depth of 1cm. Each pot was placed on an individual saucer and watered until water emerged from the base of the pot. Subsequent watering was done by filling the saucer.

The total number of emerged seedlings and those with damping-off symptoms were counted every few days from first emergence (27 February, 2, 5, 7, 9, 12 and 15 March 2012). Plant vigour was recorded on a 0-10 scale.

Results

Table 11. Emergence (%) in cultivar x pathogen experiment, 9 November 2011 (Expt. 6)

Cultivar	Seed Trt	9H1 Fus	11A1 Fus	2A1 HS	10H1 HS	1H1 P.ult	7H1 Mort	Untr	Seed trt mean
Squirrel	HDC F57	56.7	18.3	20.0	21.7	10.0	64.2	-	31.8
	HDC F58	61.7	25.8	21.7	10.8	36.7	63.3	-	36.7
	Untrt	-	-	-	-	-	-	27.5	-
Isolate/ Cultivar Mean		59.2	22.1	20.9	16.3	23.4	63.8	-	-
Swan	HDC F57	63.3	50.0	15.8	38.3	23.3	68.3	-	43.2
	HDC F58	73.3	35.8	25.0	12.5	37.5	80.8	-	44.2
	Untrt	-	-	-	-	-	-	59.2	
Isolate/ Cultivar Mean		68.3	42.9	20.4	25.4	30.4	74.6	-	-
Toucan	HDC F57	83.3	60.8	40.8	51.7	29.2	64.2	-	55.0
	HDC F58	73.3	57.5	30.0	18.3	56.7	73.3	-	51.5
	Untrt	-	-	-	-	-	-	60.0	
Isolate/ Cultivar Mean		78.3	59.2	35.4	35.0	43.0	68.8	-	

Cultivar Mean			Seed trt mean		
	Squirrel	33.7	HDC F57	43.3	
	Swan	44.9	HDC F58	44.1	
	Toucan	53.8	Untrt	48.9	

Interaction		Comparison of untreated with individual treatments		Comparison of all other treatments excluding untreated	
	FPr.	SED	LSD	SED	LSD
Cultivar	0.037	-	-	2.30	4.54
Isolate	<0.001	3.39	6.67	4.15	8.18
Seed trt	NS 0.688	1.95	3.85	3.66	7.21
Cultivar x Seed trt	NS 0.222	3.39	6.68	6.33	12.49
Isolate x Seed trt	<0.001	4.79	9.44	4.79	9.44
Isolate x Cultivar	0.024	5.86	11.56	7.18	14.16
Isolate x Seed trt x Cultivar	NS 0.215	8.29	16.36	8.29	16.36

There was greater emergence on 9 November (8 days after sowing) in Toucan and Swan than in Squirrel (Table 11). Emergence differed between the fungal isolates with *Pythium ultimum* and *Pythium* hyphal swelling isolates showing the poorest emergence. There were significant isolate x cultivar and isolate x seed treatment interactions but no seed treatment effects were significant.

Glasshouse temperatures were mostly in the range 12-16ºC (daily maximum) and 8-12ºC (daily minimum) with a cold period on 7 November (9ºC maximum, 5ºC minimum). Conditions were therefore similar to those experienced by spring and autumn crops.

Table 12. Emergence (%) in cultivar x pathogen experiment, 22 November 2011 (Expt. 6)

Cultivar	Seed Trt	9H1 Fus	11A1 Fus	2A1 HS	10H1 HS	1H1 P.ult	7H1 Mort	Untrt	Seed trt mean
Squirrel	HDC F57	56.7	20.0	30.8	16.7	10.0	68.3	-	31.7
	HDC F58	66.7	25.0	35.0	10.0	32.5	61.7	-	38.5
	Untrt	-	-	-	-	-	-	31.7	-
Isolate/ Cultivar Mean		61.7	22.5	32.9	13.3	21.3	65.0	-	35.1
Swan	HDC F57	71.7	47.5	33.3	34.1	22.5	79.2	-	68.3
	HDC F58	78.3	40.0	38.3	6.7	36.7	70.8	-	45.1
	Untrt	-	-	-	-	-	-	68.3	-
Isolate/ Cultivar Mean		75.0	43.8	35.8	20.4	29.6	75.0	-	56.7
Toucan	HDC F57	90.8	66.7	52.5	56.7	52.5	67.5	-	67.5
	HDC F58	86.7	30.0	52.5	14.2	52.5	85.0	-	61.0
	Untrt	-	-	-	-	-	-	67.5	-
Isolate/Cultivar Mean		88.8	65.8	52.5	35.4	45.2	76.3	-	64.3

Cultivar Mean	Squirrel	35.8	Seed trt mean	HDC F57 47.1
	Swan	48.3		HDC F58 48.2
	Toucan	61.2		Untrt 55.8

Interaction		Comparison of untreated with individual treatments		Comparison of all other treatments excluding untreated	
	FPr.	SED	LSD	SED	LSD
Cultivar	<0.001	-	-	3.48	6.86
Isolate	<0.001	4.10	8.08	3.34	6.60
Seed trt	NS 0.684	3.61	7.12	1.93	3.81
Cultivar x Seed trt	NS 0.273	6.26	12.34	3.34	6.60
Isolate x Seed trt	<0.001	4.73	9.32	4.73	9.32
Isolate x Cultivar	0.036	7.09	13.99	5.79	11.42
Isolate x Seed trt x Cultivar	NS 0.055	8.19	16.15	8.19	16.15

There was low emergence with four of the fungal isolates tested but Fusarium isolate 9H1 and 7H1 (*Mortierella*) appeared to have enhanced emergence (Table 12). There was no difference between seed treatments but there was an isolate x seed treatment interaction. There were significant differences between each of the cultivars and an isolate x cultivar interaction due to greater emergence with Fusarium 9H1 and Mortierella 7H1. Squirrel had the lowest germination and the addition of Fusarium 9H1 and Mortierella 7H1 almost doubled its seedling emergence (Table 12).

Table 13. Vigour (%) relative to the control in cultivar x pathogen experiment, 28 November 2011 (Expt. 6)

Cultivar	Seed Trt	9H1 Fus	11A1 Fus	2A1 HS	10H1 HS	1H1 P.ult	7H1 Mort	Untrt	Seed trt mean
Squirrel	HDC F57	420.8	100.0	64.5	85.0	30.0	387.5	-	181.3
	HDC F58	412.5	109.2	94.2	45.8	227.5	475.0	-	227.4
	Untrt	-	-	-	-	-	-	100.0	-
Isolate/ Cultivar Mean		416.7	104.6	79.3	65.4	128.8	431.3	-	204.3
Swan	HDC F57	122.5	85.8	7.0	40.8	33.3	156.7	-	74.4
	HDC F58	150.8	64.2	31.7	7.0	55.8	209.2	-	86.4
	Untrt	-	-	-	-	-	-	100.0	-
Isolate/ Cultivar Mean		136.7	75.0	19.3	23.9	44.6	182.9	100.0	80.4
Toucan	HDC F57	212.5	91.7	41.7	79.2	30.8	135.0	-	98.5
	HDC F58	198.3	135.0	45.8	18.3	135.8	225.0	-	126.4
	Untrt	-	-	-	-	-	-	100.0	-
Isolate/ Cultivar Mean		205.4	113.3	43.8	48.8	83.3	180.0	100.0	112.4

Cultivar Mean	Squirrel	196.3			HDC F57	118.0	
	Swan	81.9		Seed trt mean	HDC F58	146.7	
	Toucan	111.5			Untrt	100.0	

Interaction	FPr.	Comparison of untreated with individual treatments		Comparison of all other treatments excluding untreated	
		SED	LSD	SED	LSD
Cultivar	<0.001	-	-	20.83	41.09
Isolate	<0.001	37.56	74.08	30.67	60.49
Seed trt	NS 0.107	33.12	65.33	17.70	34.92
Cultivar x Seed trt	NS 0.736	57.37	113.16	30.67	60.49
Isolate x Seed trt	NS 0.159	43.37	85.54	43.37	85.54
Isolate x Cultivar	0.002	65.05	128.31	53.11	104.77
Isolate x Seed trt x Cultivar	NS 0.981	75.11	148.16	75.11	148.16

Using the standard compost as control, the seedling vigour was decreased by three of the pathogens but apparently enhanced by Fusarium isolate 9H1 and Mortierella 7H1 (Table 13). Pythium hyphal swelling isolates were the most damaging to seedling growth. Cultivar, isolate and isolate x cultivar interactions were significant. Squirrel was the cultivar with the most vigorous responses to seed treatment. Seed treatments did not show significant differences or any interactions.

Table 14. Emergence (%) in cultivar x pathogen experiment, 9 December 2011 (Expt. 6)

Cultivar	Seed Trt	9H1 Fus	11A1 Fus	2A1 HS	10H1 HS	1H1 P.ult	7H1 Mort	Untrt	Seed trt mean
Squirrel	HDC F57	88.1	81.4	47.4	43.2	50.0	68.6	-	63.1
	HDC F58	92.9	69.7	53.6	60.0	90.6	89.2	-	76.0
	Untrt	-	-	-	-	-	-	89.7	-
Isolate/ Cultivar Mean		90.5	75.6	50.5	51.6	70.3	78.9	-	69.6
Swan	HDC F57	79.0	67.6	13.9	53.0	72.0	81.2	-	61.1
	HDC F58	81.4	63.0	48.3	48.9	75.7	85.5	-	67.1
	Untrt	-	-	-	-	-	-	89.7	-
Isolate/ Cultivar Mean		80.2	65.3	31.1	50.9	73.9	83.3	-	64.1
Toucan	HDC F57	84.6	58.5	28.8	53.3	72.2	63.8	-	61.7
	HDC F58	75.0	76.9	48.0	58.5	95.0	73.8	-	70.3
	Untrt	-	-	-	-	-	-	77.2	-
Isolate/ Cultivar Mean		79.8	67.7	38.4	57.8	83.6	68.8	-	66.0

Cultivar Mean	Squirrel	69.6		HDC F57	62.0
	Swan	64.1	Seed trt	HDC F58	71.2
	Toucan	66.0	mean	Untrt	85.5

		Comparison of untreated with individual treatments		Comparison of all other treatments excluding untreated	
Interaction	FPr.	SED	LSD	SED	LSD
Cultivar	NS 0.434	-	-	6.39	12.60
Isolate	<0.001	7.52	14.83	6.14	12.11
Seed trt	0.010	6.63	13.08	3.54	6.99
Cultivar x Seed trt	NS 0.728	11.48	22.65	6.14	12.11
Isolate x Seed trt	NS 0.223	8.68	17.12	8.68	17.12
Isolate x Cultivar	NS 0.607	13.02	25.68	10.63	20.97
Isolate x Seed trt x Cultivar	NS 0.522	15.03	29.65	15.03	29.65

At the final assessment on 9 December (38 days after sowing), there was more emergence in Squirrel and now no significant differences between cultivars (Table 14). Low emergence with the two fungal isolates of *Pythium* hyphal swelling species continued the effects noted during the previous month. Fusarium 9H1 and Mortierella 7H1 had slightly enhanced emergence (Table 14). The *Pythium ultimum* isolate 1H1 had good emergence but gave low vigour in some of the seed treatments (Table 13). There were significant effects of seed treatment but these were due to decreased emergence (-14.3% in HDC FF58 and – 23.5% in HDC F57) compared with the untreated. Vigour assessments on 9 December were identical to those recorded on 28 November.

Table 15. Severity index (0-100) of damping-off in cultivar x pathogen experiment, 9 December 2011 (Expt. 6)

Cultivar	Seed Trt	9H1 Fus	11A1 Fus	2A1 HS	10H1 HS	1H1 P.ult	7H1 Mort	Untrt	Seed trt mean
Squirrel	HDC F57	22.4	22.2	66.1	80.3	65.7	47.5	-	50.7
	HDC F58	18.0	43.5	64.4	62.8	43.6	19.4	-	42.0
	Untrt	-	-	-	-	-	-	22.7	-
Isolate/ Cultivar Mean				32.9	65.3	71.6	54.7	33.5	-
Swan	HDC F57	39.3	51.6	89.4	72.5	52.6	34.3	-	56.6
	HDC F58	37.1	51.1	73.3	80.9	54.2	29.5	-	54.4
	Untrt	-	-	-	-	-	-	28.9	-
Isolate/ Cultivar Mean				51.4	81.4	76.7	53.4	31.9	-
Toucan	HDC F57	27.6	57.9	79.9	68.3	60.2	51.9	-	57.6
	HDC F58	39.0	39.5	69.6	72.4	32.6	47.8	-	50.2
	Untrt	-	-	-	-	-	-	43.4	-
Isolate/ Cultivar Mean				48.7	74.8	70.4	46.4	49.9	-

Cultivar Mean						
	Squirrel	44.5			HDC F57	55.0
	Swan	53.4			HDC F58	48.8
	Toucan	53.1		Seed trt mean	Untrt	31.7

		Comparison of untreated with individual treatments		Comparison of all other treatments excluding untreated	
Interaction	FPr.	SED	LSD	SED	LSD
Cultivar	<0.001	-	-	3.14	6.18
Isolate	<0.001	4.62	9.10	5.65	11.15
Seed trt	0.022	2.66	5.26	4.99	9.83
Cultivar x Seed trt	NS 0.575	4.62	9.10	9.83	17.03
Isolate x Seed trt	NS 0.269	6.53	12.87	6.53	12.87
Isolate x Cultivar	NS 0.144	7.99	15.77	9.79	19.31
Isolate x Seed trt x Cultivar	NS 0.089	11.31	22.30	11.31	22.30

There were significant differences between cultivars, fungal isolates and seed treatments in root disease severity on 9 December (Table 15). Squirrel had less severe root symptoms than Toucan and Swan when averaged over all treatments. The *Pythium* hyphal swelling isolates gave the most severe root rotting. Seed treatments resulted in more severe root disease than in the untreated. There were no significant interactions between cultivar, isolate and seed treatments.

Experiment 7: Pathogenicity of fungal isolates on different cultivars

There were significant differences in emergence between cultivars and fungal isolates. Squirrel showed very low emergence (2.5%), significantly less than Swan (31%) on 28 February (Table 16). There was no emergence in the treatment with *Pythium* hyphal swelling isolate 2A1. The Fusarium isolates appeared to enhance emergence by a factor of 2 or 3 compared with the untreated control.

Table 16. Emergence (%) in cultivar x pathogen experiment, 28 February 2012 (Expt. 7)

Cultivar	9H1 Fus	11A1 Fus	2A1 HS	10H1 HS	1H1 P.ult	7H1 Mort	Untrt	Cultivar mean
Squirrel	11.7	2.5	0.0	0.8	0.0	1.7	0.8	2.5
Swan	55.8	32.5	0.0	6.7	9.2	2.5	12.5	17.0
Toucan	59.2	44.2	0.0	31.7	37.5	20.0	24.2	31.0
Isolate mean	42.2	26.4	0.0	15.6	15.6	8.1	12.5	-

		Comparison of untreated with individual treatments		Comparison of all other treatments excluding the untreated	
Interaction	FPr.	SED	LSD	SED	LSD
Cultivar	<0.001	-	-	7.67	15.21
Isolate	<0.001	4.43	8.78	4.43	8.78
Isolate x Cultivar	<0.001	7.67	15.21	7.67	15.21

Seedling vigour relative to the untreated differed significantly between isolates and ranged from zero (i.e. no seedlings had emerged) with *Pythium* hyphal swelling group isolate 2A1 to 468 with Fusarium 9H1 (Table 17). There was a significant cultivar x isolate interaction reflecting enhanced vigour with Fusarium isolates, but no significant differences between cultivars.

Table 17. Vigour (%) relative to the untreated control in cultivar x pathogen experiment, 28 February 2012 (Expt. 7)

Cultivar	9H1 Fus	11A1 Fus	2A1 HS	10H1 HS	1H1 P.ult	7H1 Mort	Untrt	Cultivar mean
Squirrel	292	100	0	33	0	67	100	125
Swan	712	378	0	38	150	13	100	207
Toucan	399	159	0	325	204	194	100	197
Isolate mean	468	212	0	156	140	121	100	-

		Comparison of untreated with individual treatments		Comparison of all other treatments excluding the untreated	
Interaction	FPr.	SED	LSD	SED	LSD
Cultivar	0.763	-	-	49.7	98.6
Isolate	<0.001	75.9	150.6	75.9	150.6
Isolate x Cultivar	0.049	131.5	260.9	131.5	260.9

Table 18. Emergence (%) in cultivar x pathogen experiment, 26 March 2012 (Expt. 7)

Cultivar	9H1 Fus	11A1 Fus	2A1 HS	10H1 HS	1H1 P.ult	7H1 Mort	Untrt	Cultivarmean
Squirrel	16.7	5.0	0.0	1.7	0.0	5.0	12.5	5.8
Swan	64.2	38.3	0.0	9.2	11.7	9.2	35.8	24.1
Toucan	62.5	56.7	0.0	39.2	35.8	34.2	45.0	39.1
Isolate mean	47.8	33.3	0.0	16.7	15.8	16.1	31.1	-

		Comparison of untreated with individual treatments		Comparison of all other treatments excluding the untreated	
Interaction	FPr.	SED	LSD	SED	LSD
Cultivar	<0.001	-	-	3.41	6.77
Isolate	<0.001	5.21	10.34	5.21	10.34
Isolate x Cultivar	<0.001	9.03	17.91	9.03	17.91

At the final assessment on 28 March, there were significant differences in emergence between varieties and fungal isolates (Table 18). There was still no emergence in the treatment with *Pythium* hyphal swelling isolate 2A1. All cultivars had low emergence in the untreated controls, with only a few more seedlings emerging since 28 February. The *Fusarium* isolate 9H1 significantly enhanced emergence by about 50%.

Table 19. Vigour (%) relative to the untreated control in cultivar x pathogen experiment, 26 March 2012 (Expt. 7)

Cultivar	9H1 Fus	11A1 Fus	2A1 HS	10H1 HS	1H1 P.ult	7H1 Mort	Untrt	Cultivar mean
Squirrel	262	93	0	23	0	113	100	84
Swan	298	183	0	23	28	33	100	95
Toucan	216	110	0	119	61	120	100	104
Isolate mean	258	129	0	61	35	94	100	-

		Comparison of untreated with individual treatments		Comparison of all other treatments excluding the untreated	
Interaction	FPr.	SED	LSD	SED	LSD
Cultivar	NS 0.733	-	-	24.4	48.4
Isolate	<0.001	37.2	73.9	37.2	73.9
Isolate x Cultivar	NS 0.523	64.5	128.0	64.5	128.0

Seedling vigour relative to the untreated on 26 March differed significantly between isolates and ranged from zero (i.e. no seedlings had emerged) with *Pythium* hyphal swelling group isolate 2A1 to 298 with *Fusarium* 9H1 (Table 19). There was no longer a significant cultivar x isolate interaction (compare Table 17) and no significant differences between cultivars.

Seed vigour and damping-off under protected conditions

Introduction

This part of the project was undertaken to determine if the differences in damping-off between cultivars recorded in field and pot experiments were attributable to seed quality and/or cultivars. Seed stocks used earlier in the project and some new varieties were included.

Objectives

1. To evaluate varieties and seed stocks for susceptibility to damping-off diseases, particularly *Pythium* species, in soil.
2. To determine if there are differences in cultivar susceptibility to damping-off diseases.
3. To establish if damping-off susceptibility can be related to germination and vigour of spinach seed in laboratory tests.

Methods

Experiment 8

The test seed stocks were compared by sowing untreated seeds in small seed trays containing naturally infested soil under unheated polythene tunnel conditions on 3 May 2012. There were 15 seed stocks (13 cultivars) and these were grown in randomized blocks with fourfold replication. Soil was obtained on 29 March from a field at Bilsthorpe, Notts that had spinach damping-off problems in 2011 and stored in a cold store (c. 4ºC) until required. The seed trays were filled to about 70% depth with field soil) and watered to ensure it was moist. Then 50 seeds were placed in each tray evenly spaced (as 5 rows of 10 seeds) and covered with more soil to a depth of at least 1 cm. Subsequently the trays were kept moist but not excessively wet. A Tinytalk logger was used to record temperatures during the experiment. Soil pH, major nutrients and organic matter was determined on a sample taken at sowing.

Plant counts of healthy and diseased seedlings were done from early emergence of the first seedlings on 7 May at 2-4 day intervals until 28 May when no new plants emerged.

Laboratory seed tests

A minimum of 500 seeds from each seed stock was sent to Dr Graham Kinsey (Germains Seed Technology, St Andrews Road, Hardwick Industrial Estate, King's Lynn, Norfolk, PE30 4GF) for determination of thousand seed weight, germination and vigour under standard laboratory test conditions.

Table 20. Cultivars and seed stocks used in seed vigour experiment 2012 (Expt. 8)

	Cultivar	Lot number
1	Carmel	525708
2	Silverwhale	100668283/8
3	Toucan	100668290/8 (old seed)
4	Pigeon	100681184 (old seed)
5	Swan	100616009/7
6	Zebu	100592204
7	Pigeon	100671369/8
8	Toucan	100794832/4
9	Pelican	100268806/9
10	Squirrel	
11	Carmel	07218871
12	Mississippi	07223401
13	Missouri	07220711
14	Tennessee	07220044
15	Bikini (2008 unopened packet)	1525274

Results

Table 21. Mean number of emerged plants in different seed stocks during 10-18 May 2012 (Expt. 8)

	Cultivar	Number of plants/tray				
		10 May	14 May	16 May	18 May	Scheffe test
1	Carmel	3.5	4.5	2.8	4.8	a
2	Silverwhale	4.3	6.3	4.8	6.0	a
3	Toucan	5.3	12.3	10.0	13.0	ab
4	Pigeon	4.0	10.0	12.0	16.0	ab
5	Swan	0.3	0.0	0.0	0.5	a
6	Zebu	2.8	5.8	3.5	5.5	a
7	Pigeon	30.8	34.5	32.0	35.5	c
8	Toucan	19.0	27.8	25.0	27.5	bc
9	Pelican	0.3	0.3	0.5	1.8	a
10	Squirrel	0.3	0.0	0.0	0.5	a
11	Carmel	25.0	30.8	24.8	26.0	bc
12	Mississippi	0.5	3.8	5.0	6.0	a
13	Missouri	1.5	6.5	9.0	12.0	ab
14	Tennessee	3.3	5.3	5.0	7.5	a
15	Bikini	1.5	5.8	6.5	8.8	a
	Fpr.	<0.001	<0.001	<0.001	<0.001	
	Sed	2.65	3.24	3.29	3.29	
	Lsd	5.34	6.54	6.65	6.65	

Most seed stocks gave low plant emergence on 10 May (7 days after sowing) and Pigeon, Carmel and Toucan had significantly more emergence than all the other stocks (Table 21). Plant counts up to 18 May showed there was some slow, continuing emergence. The Scheffe test on data for 18 May is included to show individual stocks differed from each other (cultivars with the same letter do not differ from each other).

Further plant counts up to 28 May showed some decreases in plant numbers (see also Fig. 5) as damping-off caused small seedlings to die and disappear as they shrivelled (Table 22). Differences between seed lots were significant but smaller than in the initial counts.

The mean percentage of seedlings with damping-off was 58% on 14 May and this declined over subsequent assessments to 28% on 16 May, 34% on 18 May and 12% on 21 May. The number of healthy plants per tray peaked at 8.2 on 21 May and then decreased to 5.8 plants/tray by 28 May as damping-off caused more plant losses.

Table 22. Mean number of emerged plants in different seed stocks 21-28 May 2012 (Expt 8)

	Cultivar	Number of plants/tray		
		21 May	25 May	28 May
1	Carmel	2.5	2.5	2.8
2	Silverwhale	3.8	3.8	3.5
3	Toucan	10.3	10.3	9.0
4	Pigeon	14.0	14.5	14.8
5	Swan	1.5	1.5	1.5
6	Zebu	2.5	2.3	2.3
7	Pigeon	22.0	18.3	17.8
8	Toucan	20.3	16.0	15.8
9	Pelican	5.0	5.8	5.3
10	Squirrel	2.0	3.5	2.5
11	Carmel	20.8	16.8	15.3
12	Mississippi	7.5	7.3	7.5
13	Missouri	13.0	12.8	12.0
14	Tennessee	6.5	8.3	8.3
15	Bikini	7.3	8.8	7.8
	Fpr.	<0.001	<0.001	<0.001
	Sed	3.402	3.542	3.547
	Lsd	6.866	7.148	7.157
Repeated measures results				
Seedlot	Fpr.	<0.001		
	Sed	2.97		
	Lsd	5.99		
Seedlot xTiming	Fpr.	<0.001		
	Sed	3.31		
	Lsd	7.39		

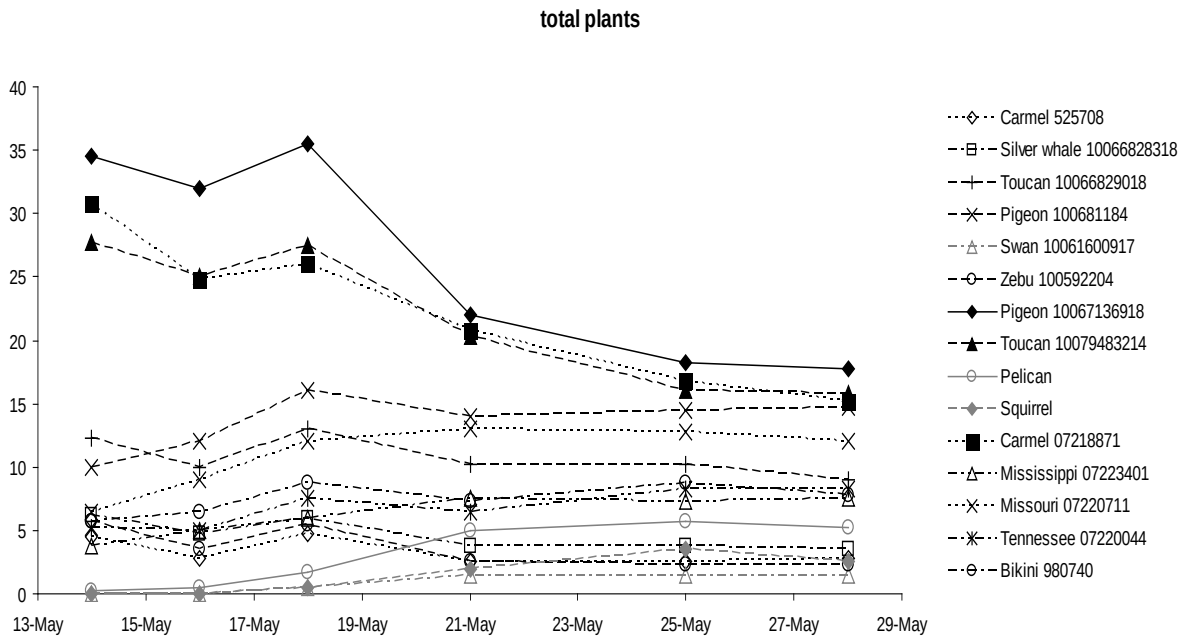

Figure 5. Changes in plant numbers in various seed stocks over time, May 2012 (Expt. 8)

Statistically there were at least two major groups for germination with the Pigeon 10067136918, Toucan 10079483214 and Carmel 07218871 (solid symbols) showing better early emergence. Swan, Pelican and Squirrel (grey symbols) showed a later germinating pattern (Fig. 5).

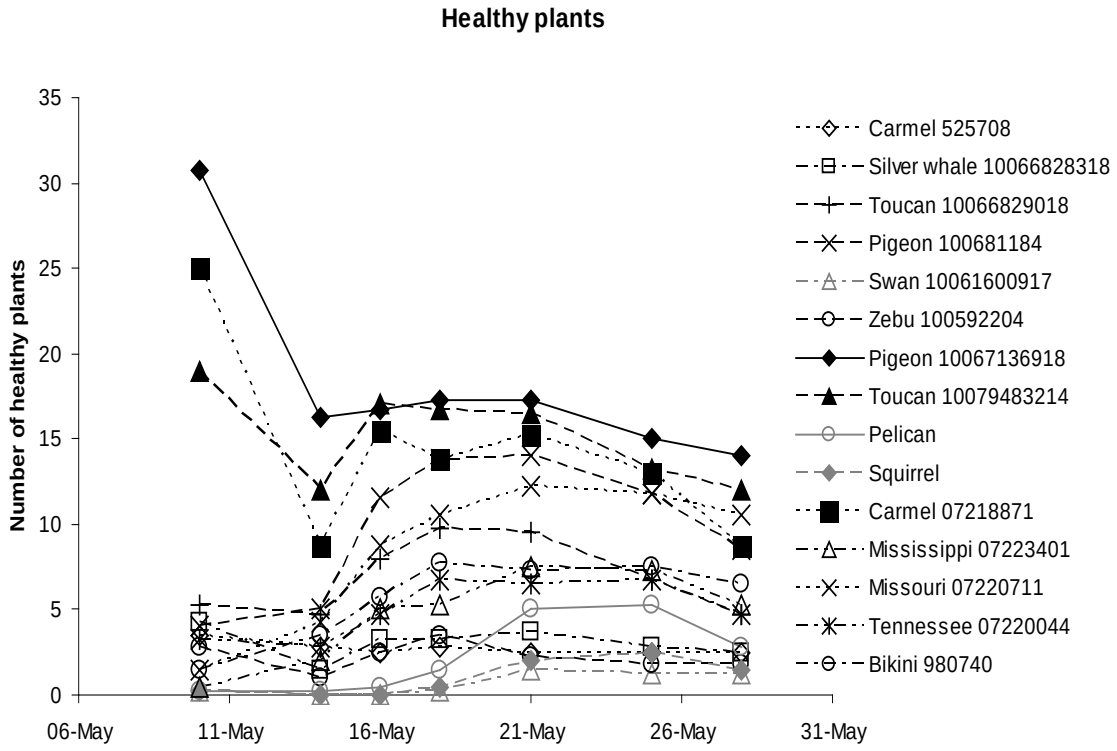

Figure 6. Changes in the number of healthy plants in various seed stocks over time, May 2012 (Expt. 8)

Plants became less healthy as time progressed and damping-off affected a high proportion of seedlings that emerged. *Pythium* was confirmed as the main pathogen causing damping-off by microscopic examination of affected seedlings. Pigeon 100681184, Toucan 10079483214 and Carmel 07218871 had the greatest number of healthy plants at the beginning of the experiment (Fig. 6). By the end of the experiment, there was only a limited difference between them and Pigeon 10067136918 or Missouri.

The proportion of the maximum emerged plants which have damping-off (mainly wilted plants) varied during the course of the experiment (Fig. 6). The initial levels of damping-off tended to decrease up to 21 May and then increased up to the end of the experiment. This reflected a balance between new plants emerging and disease progress. Damping-off affects seedlings as they germinate so poor emergence is also likely to be due in part to a pre-emergence phase of damping-off in these experiments. Actual germination levels in the absence of damping-off are provided by the laboratory seed tests (see Table 24).

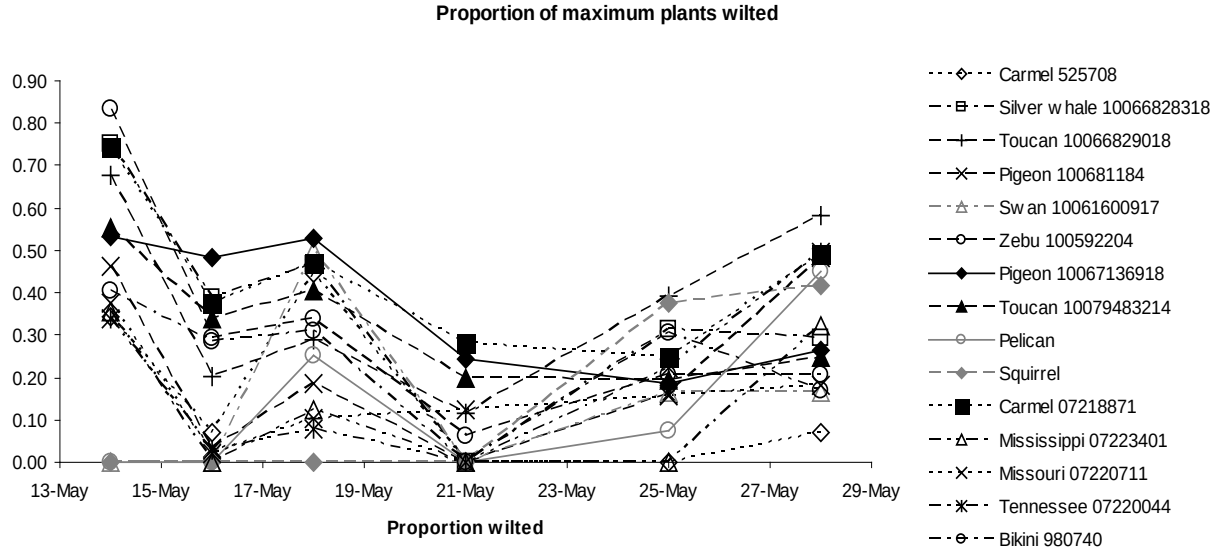

Figure 7. Proportion of plants with damping-off (wilting symptoms) in various seed stocks over time, May 2012 (Expt. 8)

On 10 and 14 May, differences in numbers of healthy plants (range 0.3 to 30.8 plants per tray) were significant in the anova analyses and the Scheffe test identified the various seed stocks with similar plant counts (Table 23). From 18 May onwards, there were no differences between seed lots in mean healthy plant numbers. There were significant differences between seed lots when data were examined across assessment dates and a seed lot x assessment timing interaction (Table 23).

Table 23. Mean number of healthy plants in different seed stocks during 10-16 May 2012 (Expt. 8)

	Cultivar	Number of plants/tray		
		10 May	14 May	16May
1	Carmel	3.5 a	2.8ab	2.5
2	Silverwhale	4.3a	1.5ab	3.3
3	Toucan	5.3ab	4.8ab	8.0
4	Pigeon	4.0a	5.0ab	11.5
5	Swan	0.3a	0.0a	0.0
6	Zebu	2.8a	1.0a	2.5
7	Pigeon	30.8a	16.3b	16.
8	Toucan	19.0bc	12.0b	17.0
9	Pelican	0.0a	0.3a	0.5
10	Squirrel	0.3a	0.0a	0.0
11	Carmel	25.0c	8.8ab	15.5
12	Mississippi	0.5a	2.0ab	5.0
13	Missouri	1.5a	4.3ab	8.8
14	Tennessee	3.3a	2.8ab	4.8
15	Bikini	1.5a	3.5ab	5.8
	Fpr.	<0.001	<0.001	NS
	Sed	2.648	3.497	
	Lsd	5.344	7.058	
Repeated measures results				
Seedlot	Fpr.	<0.001		
	Sed	2.721		
	Lsd	5.492		
Seedlot xTiming	Fpr.	<0.001		
	Sed	3.349		
	Lsd	7.695		

Laboratory seed tests

The seed test results showed highly significant differences between seed lots for germination and abnormal seedlings (Table 24) and for germination rate (Table 25). Germination was low (78-80%) in Squirrel and Zebu, whilst all other seed lots had at least 90% germination. Abnormal seedlings were most evident in Squirrel, Zebu, one lot of Carmel and Bikini and these four batches plus Mississippi had less than 90% normal germination (Table 24). The means were compared using a Scheffe test with letters indicating where seed stocks differed from each other.

Table 24. Laboratory seed germination test results on seed batches in Expt. 8, May 2012

Cultivar	Germination (%)		Abnormals		Germination less abnormals	
	Mean	±SE	Mean	±SE	Mean	±SE
Carmel	95.3c	1.2	11.3bc	0.9	84.0bc	1.0
Silverwhale	98.3c	0.3	1.3a	0.3	97.0c	0.0
Toucan	96.3c	0.3	4.0ab	0.6	92.3c	0.3
Pigeon	98.7c	1.3	2.0a	0.6	96.7c	1.5
Swan	95.3c	0.3	3.3ab	0.3	92.0c	0.6
Zebu	80.0ab	4.0	8.7bc	1.7	71.3ab	2.4
Pigeon	99.3c	0.7	2.3a	0.9	97.0c	0.6
Toucan	94.7c	0.3	2.7ab	1.5	92.0c	1.2
Pelican	94.0c	1.7	4.0ab	1.0	90.0c	1.0
Squirrel	77.7a	4.4	14.0c	2.6	63.7c	6.2
Carmel	96.3c	1.3	4.7ab	1.2	91.7c	2.0
Mississippi	90.0abc	1.5	4.0ab	0.6	86.0c	1.0
Missouri	93.7c	2.4	2.0ab	1.0	91.7c	1.5
Tennessee	92.7bc	1.2	1.3a	0.3	91.3c	1.5
Bikini	94.7c	1.5	7.0abc	1.2	87.7c	0.3
Fpr.	<0.001		<0.001		<0.001	
Sed	2.427		1.637		2.690	
Lsd	4.972		3.352		5.509	

Table 25. Laboratory seed germination rate results and thousand seed weights of seed batches used in Expt. 8, May 2012

Cultivar		Time to reach 50% germination (hours)		TSW (g)
		Mean	±SE	
1	Carmel	60.3bcd	1.1	12.3
2	Silverwhale	56.7abc	0.1	16.0
3	Toucan	62.4bcd	0.5	13.7
4	Pigeon	78.7ef	1.0	12.8
5	Swan	80.4f	1.6	15.3
6	Zebu	66.6cde	1.8	13.9
7	Pigeon	44.4a	0.4	13.0
8	Toucan	51.7ab	0.7	10.7
9	Pelican	108.8g	1.5	11.0
10	Squirrel	127.4h	5.7	13.0
11	Carmel	45.5a	0.8	11.3
12	Mississippi	78.8ef	0.8	9.8
13	Missouri	80.0ef	0.7	16.9
14	Tennessee	80.4f	1.0	20.3
15	Bikini	73.0def	0.8	6.5
	Fpr.	<0.001		
	Sed	2.562		
	Lsd	5.248		

There was a large range in thousand seed weights from 6.5 g in Bikini to 16.0 g with Silverwhale (Table 24). The time to reach 50% germination varied from 44 h in Pigeon to 127 h with Squirrel (Table 25). The differences in germination times were highly significant.

Correlations between seed test results were significant for the negative impact of abnormal plants on the number of healthy plants and for abnormal plants on the 10 day germination count. The rate of germination (t50) was negatively correlated with the healthy plant count and the number germinated (Table 26).

Table 26. Correlation matrix for seed test parameters (data from Tables 24 and 25)

Abnormal seeds	1	-			
Healthy plants	2	-0.8742***	-		
Number germinated (10 day count)	3	-0.6936**	0.9561***	-	
T50	4	0.3956	-0.5496*	-0.5768*	-
TSW	5	-0.3225	0.1217	-0.0139	0.0496
Factor		Abnormal seeds	Healthy	Number germinated	T50

Correlation P <0.001 *** ; P<0.05*

Correlations with seed test results and data from the tray tests (Expt. 8) identified significant negative relationships with germination rate (t50) and the total and healthy plant counts on 18 May (*r* = -0.68 and -0.62 respectively). Germination rate was also negatively correlated with the number of emerged plants on 10 May May (*r* = -0.67) and positively with the proportion of plants with damping-off on 18 May May (*r* = 0.62). Thus, slow germinating stocks are more prone to damping-off.

Discussion

Significant levels of damping-off developed in both field and pot experiments. Pathogenicity tests confirmed that *Pythium* spp. were important pathogens. Growers need to be aware that other pathogens can affect spinach seedlings and may be more important than *Pythium* spp. on some sites. These include *Rhizoctonia* spp., *Aphanomyces cochlioides* and even *Pleospora bjoerlingii* (= *Phoma betae*) (Koike *et al*., 2007). *A. cochlioides* is most damaging when soil temperatures are above 15ºC and might affect crops sown in late spring or summer.

The *Fusarium* isolates and *Mortierella* isolate represent common soil fungi that can grow quickly and colonise damaged roots. Once established, *Fusarium* spp. in particular may be ableto cause further root damage and impair the growth of seedlings that are not killed outright by *Pythium* spp.

The growth enhancement observed from inoculation of the *Fusarium* isolates and *Mortierella* isolate in the pathogenicity tests was unexpected. There poor emergence in the standard

John Innes compost suggesting that pathogenic organisms were present. Thus the enhancement could result from suppression of organisms or represent a response to the inoculum itself (in the absence of direct damage observed where *Pythium* spp. were inoculated. Further testing might be considered to establish if soils could be inoculated with non-pathogens to protect seedlings against damping-off pathogens.

It was disappointing to find that various seed treatments and pre-emergence sprays to soil were not effective against *Pythium* spp. This does confirm why problems continue to occur in commercial crops when weather conditions are favourable for damping-off. The main damage appears to occur within a few days of sowing so candidate treatments must be able to work as soon as the seed is sown. This presents a stern challenge particularly for biological control treatments as propagules delivered on seed may need to germinate and grow before they can give control. Interest in pre-planting soil treatments with amendments or biological control agents may therefore be worthy of further development. Novel fungicide products or mixtures may become available in future though products of current interest have now largely been considered.

The field and pot experiments identified significant differences between cultivars in their susceptibility to damping-off. This was followed up by comparing the features of different seed stocks though we did not compare more than two stocks of any one cultivar. There were large differences in thousand seed weight between seed lots and in speed of germination. Seed lots with high germination in the laboratory may still show severe losses from damping-off. However, early vigour (time to 50% germination) appears to be a positive feature that may be beneficial for plant populations at sites with damping-off. Further work is required to establish if there are differences in susceptibility to *Pythium* spp. Data from this project suggest that seed quality is an important factor to consider.

The options to control damping-off under field conditions are limited. Damping-off pathogens such *Pythium* spp. and *Rhizoctonia* spp. are widely distributed in UK. Clearly the risk at individual sites is likely to vary based on previous cropping and management. Risk assessment and pre-planting soil tests may help identify high risk sites that are avoided or only used when weather patterns are judged settled and unfavourable. This is clearly difficult to manage for large scale intensive production systems.

The results from spinach may also be of benefit to related crops such as chard and red beet. For example an EAMU (0526/2012) has recently become available for the use of Apron XL on chard seed. In general, seed treatments should be used as part of an integrated strategy including careful site selection.

Conclusions

Occurrence

- Following a relatively dry spring and summer 2010, outbreaks of damping-off were not reported until autumn 2010. Problems were associated with high rainfall and areas were watered accumulated. Badly affected areas showed 70-80% plants affected.
- There were more cases of damping-off in commercial crops in 2011. These affected only some of the sowings in an individual field which was attributed to variation in weather at or soon after sowing.
- Damping-off was evident from emergence onwards. Seedlings often collapsed at the cotyledon stage and the incidence of damping-off decreased as plants became larger.

Crops and cropping history

- Damping-off occurred on a range of cultivars and despite use of seed treatments. There was no obvious association with previous crop rotations though the number of case histories examined was small.
- There were significant differences in damping-off between cultivars though this may be due to differences in seed lots rather than to differences in cultivar susceptibility.

Cause

- *Pythium* and *Fusarium* species were frequently isolated and appear to be the mostly likely pathogens. However, spinach crops can be affected by various other pathogens and growers should continue to seek identification of the cause of seedling losses.
- *Pythium* species were found to be the main pathogens causing damping-off of spinach in this project.
- Pathogenicity was demonstrated for *Pythium* isolates but other fungi recovered from roots, particularly Fusarium spp. appeared to cause little or no damage.
- *Fusarium* isolates proved to be capable of causing leaf infection.
- Pythiums assigned to the Hyphal Swellings Group and *Pythium ultimum* were identified in various crops. Initial pathogenicity tests did not show significant effects on germination or seedling vigour compared to untreated control.

Control

- Seed treatments and pre-emergence fungicide sprays did not control damping-off.
- A comparison of various seed lots suggested that speed of germination (time to reach 50% germination) was a beneficial characteristic that improved plant survival when there was damping-off.
- Growers have few options available to control damping-off and site selection should therefore be given high priority.

Technology transfer

- Visits to spinach growers (Kent and Nottinghamshire) in September 2010
- Short article for *HDC News*, June 2011
- Article for *HDC News*, July 2012

References

Gladders P. 2008. Baby leaf crucifers: improving control of downy mildew. HDC Project FV 316. Annual Report.

Kinsey G. 2009. A new treatment for spinach seed with efficacy against seed- and soil-borne fungal pathogens, in particular *Verticillium dahliae*. BCPC Seed Treatments Conference, 2009, pp 31-36.

Koike ST, Gladders P & Paulus AO. 2007. *Vegetable Diseases: A Colour Handbook*. London: Manson Publishing.

Larsson M. 1994. Prevalence and pathogenicity of spinach root pathogens of the genus *Pythium* in Sweden. *Plant Pathology*, **43:** 261-268.

Larsson, M. & Gerhardson, B. 1992. Disease progression and yield losses from root diseases caused by soilborne pathogens of spinach. *Phytopathology* **82**: 403-406.

Larsson, M. & Olofsson, J. 1994. Prevalence and pathogenicity of spinach root pathogens of the genera *Aphanomyces*, *Phytophthora*, *Fusarium, Cylindrocarpon*, and *Rhizoctonia* in Sweden. *Plant Pathology*, **43**: 251-260.

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Appendix 1

Agar preparation

1. PDA + S

To make 1 litre

- Autoclave 39 g potato dextrose agar (PDA) in 1 L distilled water
- Cool to 50°C
- Make a stock solution of 1.25 g streptomycin sulphate in 500 ml sterile distilled water
- Add 10 ml stock solution to 1 L agar using a sterile pipette. Swirl agar to mix

2. P_5ARP AGAR

To make 1 litre

- Autoclave 17 g Difco Corn Meal Agar (CMA) in 1 L distilled water
- Cool to 50 °C
- Add 5 mg/L picmaricin
- 250 mg/L ampicillin
- 10 mg/lL rifampicin
- 100 mg/L PCNB

To make up the required amounts to go in 1L

Add with sterile syringes and swirl agar well to mix

Pimaricin (in fridge)

100 mg pimaricin in 20 ml sterile distilled water = 5 mg/ml in stock solution

Add 1 ml to 1 L agar to give 5 mg/L

Ampicillin

560 mg ampicillin in 11.2 ml sterile distilled water = 50 mg/ml in stock solution

Add 5 ml to 1 L agar to give 250 mg/L

Rifampicin

100 mg rifampicin in 20 ml 96% ethanol = 5 mg/ml in stock solution

Add 2 ml to 1 L agar to give 10 mg/L

PCNB

2g of Quintozine (pentachloronitrobenzene) (99% w/w) in 20 ml sterile distilled water

= 100 mg/ml in stock solution

Add 1 ml to 1 L to give 100 mg/L

Excess made-up volumes can be stored in the fridge for about a week.
All except PCNB purchased bottles should be in the fridge

3. Semi-selective medium for *Fusarium* species (SMF)

To 1 l distilled water add:
20 g Technical agar
23 g Glucose
5.0 g KNO_3
2.5 g KH_2PO_4
1.2 $MgSO_4$
12.5 mg metalaxyl*

- Autoclave
- Cool to 50 °C

Add 100 mg streptomycin (from stock solution)

*From Subdue fungicide (465.2 g metalaxyl per L)
Take 1 ml Subdue and dilute in 100 ml
- gives 4.652 mg metalaxyl per ml
- so 2687 ul (2.687 ml) gives 12.5 mg ai

4. Selective medium for *Aphanomyces* species (SMA)

To 1 l distilled water add:
10 g cornmeal agar
10 g technical agar
12.5 mg metalaxyl
5 mg benomyl

- Autoclave
- Cool to 50 °C

Add 100 mg streptomycin sulphate (from stock solution)

References for SMA and SMF media:

Larsson M & Olofsson J. 1994. Prevalence and pathogenicity of spinach root pathogens of the genera *Aphanomyces*, *Phytotophthora*, *Fusarium*, *Cylindrocarpon*, and *Rhizoctonia* in Sweden. *Plant Pathology* **43**: 251-260.

Appendix 2

Compost analysis: John Innes Seeding compost (Expts 6 and 7)

Factor	Value	Factor	Value
pH	6.61	Cond. at 20°	324 uS/cm
Density	788 kg/m^3	Ammonia-N	43.5 mg/l
Dry matter	58.5%	Nitrate-N	123.4 mg/l
Dry density	461 kg/m^3	Total soluble N	166.9 mg/l
Chloride	31.3 mg/l	Sulphate	367.9 mg/l
Phosphorus	6.5 mg/l	Boron	<0.06 mg/l
Potassium	155.0 mg/l	Copper	<0.06 mg/l
Magnesium	46.5 mg/l	Manganese	0.09 mg/l
Calcium	126.9 mg/l	Zinc	<0.06 mg/l
Sodium	36.5 mg/l	Iron	0.91 mg/l