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Horticultural Development Council

Spinach: biology and integrated management of leaf spot – phase II

FV 268a

March 2007

Project title:	Spinach: biology and integrated management of leaf spot – Phase II
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The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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CONTENTS

GROWER SUMMARY

Headline

- Two fungal pathogens that cause leaf spots in spinach (*Stemphylium botryosum* and *Cladosporium variabile)* were detected in several seed lots of spinach varieties used for UK baby leaf production.

- *Colletotrichum dematium,* the cause of the disease - spinach anthracnose, was not detected in spinach seed but was found to survive between growing seasons on volunteer spinach plants.

- Of the seven foliar fungicide and novel products evaluated in inoculated pot trials, Signum (boscalid + pyraclostrobin) was found to be the most effective fungicide for control of spinach anthracnose and Cladosporium leaf spot.

Background and expected deliverables

Major UK producers and crop consultants report that leaf spot diseases are becoming an increasing constraint to the production of baby- and mid-leaf spinach crops for which there is zero tolerance of leaf blemishes. Findings from Phase I of the project (FV 268) demonstrated that spinach leaf spots in the UK can be caused by a range of pathogens and the relative importance of different pathogens may vary between fields and seasons. Key pathogens recorded in the 2005 UK growing season were *Stemphylium*, *Cladosporium* and *Colletotrichum* species. The overall objective of Phase II of this project is to provide a clearer understanding of the biology of key leaf spot pathogens identified in Phase I, and to test options for integrated disease management.

The specific objectives are to:

1. Determine the incidence of three common leaf spot pathogens in commercial seed lots of baby leaf spinach varieties used in the UK.
2. Investigate the role of different sources of inoculum (seed and crop debris) in the development of leaf spot diseases in UK baby leaf spinach production.
3. Investigate the effect of environmental conditions (temperature, leaf wetness and relative humidity) on infection and leaf spot development using three selected pathogens.
4. Determine whether infection of spinach by one foliar pathogen facilitates infection by others.

5. Compare the susceptibility of widely used baby-leaf spinach varieties to three selected leaf spot pathogens.
6. Screen foliar fungicides and other novel products for their relative protectant and eradicant activity against three spinach leaf spot pathogens, in inoculated pot trials.
7. Evaluate promising products for the control of spinach leaf spot diseases in an inoculated field experiment.
8. Produce a Factsheet update to include project results and outline a strategy for integrated management of spinach leaf spots in the UK.

Summary of the project and main conclusions

The project focusses on three leaf spot pathogens found to occur on spinach in the UK (Final report, project FV 268), *Colletotrichum dematium* (spinach anthracnose), *Cladosporium variabile* (Cladosporium leaf spot) and *Stemphylium botryosum* (Stemphylium leaf spot). This report describes work completed in year 1 of the project, including studies on sources of inoculum, fungicide efficacy in inoculated experiments and varietal susceptibility.

Sources of inoculum

Seed

An experiment was done to determine the incidence of leaf spot pathogens in spinach seed lots of varieties used for baby leaf production in the UK. A preliminary assay was done using fungicide-treated seed of two varieties and subsequently untreated seed of eight varieties was assayed.

Stemphylium botryosum (Stemphylium leaf spot) and *Cladosporium variabile* (Cladosporium leaf spot) were detected in seed lots of spinach varieties used for UK baby leaf production. *S. botryosum* was most prevalent, being found in 9 out of 10 seed lots, with percentage incidence ranging from 0.8 to 27%. *Cladosporium variabile* was present in two out of ten seed lots at low levels (1.3% or less). *Colletotrichum dematium* (spinach anthracnose) was not detected in any of the seed lots suggesting that it is present at very low levels or absent from seed. Other sources of inoculum may therefore be responsible for outbreaks of this disease in the UK. *Verticillium dahliae* was also detected in six out of eight spinach seed lots tested. Although this pathogen does not affect spinach grown for fresh and processing use, its presence on seed is of concern since it may affect other crops in the rotation, emphasising the need for an effective seed treatment.

Volunteer plants

Leaf lesions were observed on volunteer spinach plants from a commercial holding in February 2007. The grower reported that the plants originated from a part of a field where crop destruction by herbicide at the end of the previous autumn (2006) had been incomplete. The plants had re-sprouted and showed abundant leaf spotting. There was concern that the volunteer plants could act as a source of inoculum for disease on new crops planted in February.

Laboratory examination confirmed that the lesions were due to *C. dematium*, cause of spinach anthracnose. Viable spores of the pathogen were present within leaf lesions on the volunteer plants. In the field, spores of *Colletotrichum* species are readily dispersed by rain splash and therefore infected plants could pose a risk to nearby spinach crops emerging in spring. The study confirmed that *C. dematium* can overwinter on volunteer spinach in the UK. The infected plant debris was stored in soil to be used in a further study to determine whether disease transmission can occur from infested soil to spinach seedlings.

Fungicide efficacy

Artificially inoculated experiments were done to determine the efficacy of fungicides applied at different timings in relation to infection, for control of spinach leaf spots caused by *Colletotrichum dematium*, *Cladosporium variabile* and *Stemphylium botryosum.* The crop safety of the products used was also monitored. Sprays were applied to spinach plants in seed trays.

Of seven fungicide and novel products tested, Signum (boscalid + pyraclostrobin) provided the most consistent control of two leaf spot pathogens, *C. dematium* and *C. variabile*, with opportunity for disease control when applied up to 3 days before, or 1 day after an infection event (Figures 1.1 and 1.2). Of other products currently approved for spinach, Teldor (fenhexamid) did not provide adequate disease control. Of products not currently permitted on spinach or not marketed as fungicides, Amistar (azoxystrobin) was effective for disease control but occasionally phytotoxic (in agreement with grower observations). Switch (cyprodonil + fludioxonil), Folicur (tebuconazole), Plover (difenoconazole) and Pre-Tect (Harpin) each provided excellent control of one pathogen but not both. Pre-Tect contains micronutrients and a protein (Harpin) initially isolated from a bacterium that is reported to trigger plant biochemical pathways that stimulate certain growth and stress-defence responses. An experiment to test efficacy of the same range of products against Stemphylium leaf spot is ongoing.

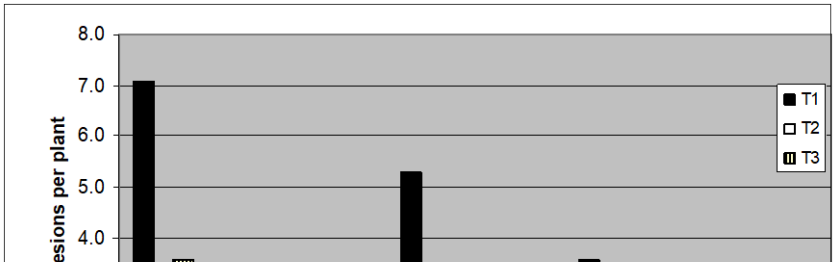

Figure 1.1. Efficacy of products for control of *Colletotrichum dematium* (spinach anthracnose) when applied 3 days before (T1), 1 day before (T2) or 1 day after (T3) inoculation

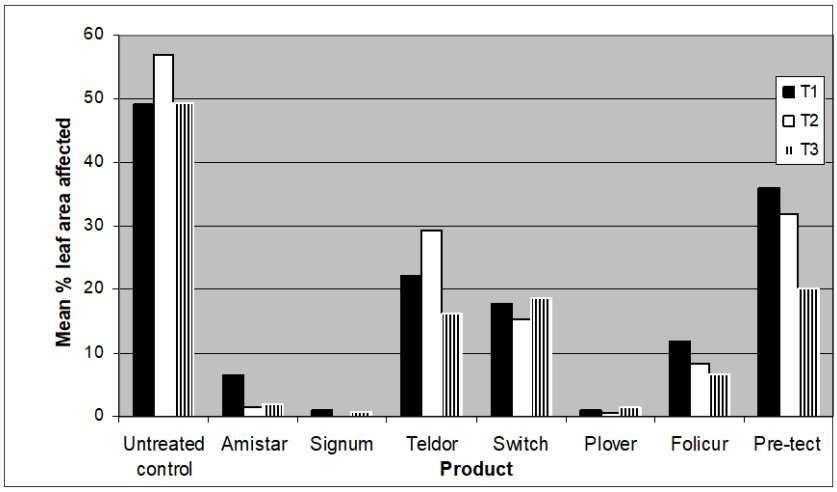

Figure 1.2. Efficacy of products for control of *Cladosporium variabile* (Cladosporium leaf spot) when applied 3 days before (T1), 1 day before (T2) or 1 day after (T3) inoculation

Varietal susceptibility

Twelve spinach varieties used for UK baby leaf production were tested for susceptibility to the leaf spot pathogens *C. dematium* (spinach anthracnose) and *C. variabile* (Cladosporium leaf spot). The experiments were done on plants with at least two true leaves, under high inoculum pressure to ensure disease development. In all experiments, the uninoculated plants remained symptom-free, indicating that disease development was as a result of artificial inoculation, rather than seed-borne inoculum.

None of the spinach varieties tested were resistant to the leaf spot pathogens *C. dematium* (spinach anthracnose) or *C. variabile* (Cladosporium leaf spot). However, for some varieties, there was consistency in their level of susceptibility to one or both pathogens (Table 1.1).

Varieties that showed lower susceptibility to a single pathogen were Matisse (versus *C. dematium*) and Tarpy (versus *C. variabile*). RZ 51-309 and Monza appeared most promising as varieties with lower susceptibility to both pathogens. Lazio was highly susceptible to both spinach anthracnose and Cladosporium leaf spot, and this finding was in agreement with grower observations.

Table 1.1. Ranked susceptibility of 12 spinach varieties to two leaf spot pathogens, following artificial inoculation using spore suspension

	Variety	Ranking in susceptibility to *C. dematium**		Ranking in susceptibility to *C. variabile**		Overall ranking
		Run 1	Run 2	Run 1	Run 2	
1	Lazio	10	12	11	12	12
2	Monza	=4	2	3	2	2
3	Matisse	1	1	6	8	3
4	Swan	6	4	4	=4	4
5	Pelican	7	10	7	=6	8
6	Bison	3	3	12	10	7
7	Buffalo	9	=6	8	11	9
8	Emelia	12	11	5	=8	11
9	Tarpy	8	9	2	3	5
10	Grizzly	11	8	10	=6	10
11	RX 06642084	=4	=6	9	=4	6
12	RZ 51-309	2	5	1	1	1

*1=least susceptible, 12 = most susceptible

Financial benefits

Producers of baby- and mid-leaf spinach are in agreement that leaf spots are increasingly a major constraint to production. For example one major grower reported 15% of drilled area affected with leaf spot diseases in 2003.

It is intended that the industry will benefit through reduced losses due to spinach leaf spot, achieved through:

- Increased knowledge of the identify and biology of pathogens causing spinach leaf spot
- Information on fungicide efficacy against selected leaf spot pathogens, possibly leading to new SOLA applications.
- Improved options for reducing fungicide usage through the use of cultural (and other non-chemical control) methods for management of spinach leaf spot.

Given that some pathogen problems (e.g. Stemphylium leaf spot) have been recorded on samples from both UK, Spain and Portugal, project results will be of relevance to both home and overseas production.

Action points for the industry

- Be aware of the range of symptom types typical of spinach leaf spot pathogens. See HDC Factsheet 08/06.
- Key leaf spot pathogens (*Stemphylium botryosum* and *Cladosporium variabile*) can be seed-borne. Check the health status of seed before use.
- Spinach anthracnose (*Colletotrichum dematium*) can overwinter on volunteer spinach plants. Ensure that plants are properly killed (e.g. by herbicide application) between crops and seasons.
- Under high risk conditions for leaf spot development, an application of Signum (boscalid + pyraclostrobin) (following the SOLA conditions of use) can provide effective disease control of spinach anthracnose and Cladosporium leaf spot, without phytotoxicity.

SCIENCE SECTION

Introduction

Phase I of this project identified key pathogens causing leaf spot diseases of spinach in the 2005 season. These were *Stemphylium botryosum*, *Colletotrichum dematium* f. sp. *spinaciae* and *Cladosporium variabile*. *S. botryosum* was most frequently isolated from spinach samples received from the UK and Spain. However, leaf spots caused by *C. dematium* (spinach anthracnose) and *C. variabile* were also observed on samples sent from UK sites. These three pathogens have all previously been recorded as causing leaf spot disease of spinach, although prior to this project there were no official records of *S. botryosum* on spinach in the UK (C. Lane, CSL, pers. comm.).

Following a knowledge review completed in Phase I of the project (Final Report, FV 268), gaps in knowledge on the biology and management of spinach leaf spot in the UK were identified as follows:

- Although the seed-borne nature of *S. botryosum*, *C. dematium* and *C. variabile* has been confirmed, levels of these pathogens in seed lots of varieties used for baby leaf spinach production in the UK have not been established.
- The importance of seed-borne inoculum for the development of spinach anthracnose (*C. dematium*) has not been confirmed.
- Studies in the USA confirmed that *Stemphylium* could survive in woody debris from spinach seed crops. However, the role of infected crop debris and volunteer plants in the development of leaf spot diseases in intensive baby-leaf spinach production needs to be ascertained.
- It is not known whether the risk of leaf spot diseases developing is increased by re-use of crop meshes. This topic is the subject of a separate project (FV 283).
- Lesions caused by downy mildew and different leaf spot pathogens can occur on the same plant. Studies are needed to confirm whether infection by one pathogen increases plant susceptibility to infection by other pathogens, or whether there is a synergistic effect of pathogen combinations.
- The general environmental conditions favouring development of different leaf spot diseases have been reported. More precise information is now required to enable high and low risk periods to be identified that may impact on irrigation scheduling, or timing of fungicide applications. It is also hypothesised that sharp drops in temperature (typical of UK autumn conditions) could render plants more susceptible to leaf spots.

- UK growers observe that leaf spots are more severe on certain spinach varieties than others and in some cases have to abandon particular varieties for this reason. Further information is required on the relative susceptibility of varieties widely used for baby leaf production in the UK.
- Further information is needed on fungicide activity against different leaf spot pathogens and optimum timing in relation to infection events. Some growers are trialling novel products (e.g. *Bacillus subtilis* and Harpin) for the control of spinach downy mildew. The management of leaf spot diseases using novel products also warrants study.

The overall aim of this project is to reduce losses due to spinach leaf spots by providing a clearer understanding of the biology of three key leaf spot pathogens identified in Phase I and by devising integrated disease management strategies.

This report describes work completed in year 1 of the project, focussing on sources of inoculum, varietal susceptibility and fungicide efficacy in inoculated experiments.

Culturing leaf spot pathogens

Experimental work focussed on three leaf spot pathogens found to occur on spinach in the UK (Final report, project FV 268), *Colletotrichum dematium*, *Cladosporium variabile* and *Stemphylium botryosum*. Different techniques were used for culturing and producing inoculum of the different pathogens. *C. dematium* grew readily on potato dextrose agar amended with streptomycin (PDA+S). However, for production of conidia, ¼ strength PDA+S was found to be a better agar medium. Placing plates of the fungus under UV lights also encouraged abundant sporulation. Cultures of *C. variabile* grew and sporulated well on PDA+S incubated in the dark. *S. botryosum* grew easily on a range of agar media including PDA+S and malt extract agar, but frequently reverted to the teleomorph phase in which abundant pseudothecia were produced in culture rather than conidia. A range of techniques was tested to encourage sporulation. For example, pieces of sterile filter paper (1 cm^2) were placed on the surface of cultures of *S. botryosum* both on PDA+S and tap water agar (P. Beales, pers. comm.). Spore production did develop on the filter paper sections, particularly in cultures on tap water agar, but this tended to be sparse. The most successful technique was to culture the fungus on V8 agar (200 ml V8 juice, 20 g agar powder, 3 g calcium carbonate in 1 L water) and to incubate plates at approximately 20^{o}C under 12 h light/12 h dark, with plates left unsealed (L. du Toit, pers. comm.).

Sources of inoculum

Although the seed-borne nature of *S. botrysosum*, *C. dematium* and *C. variabile* has been confirmed (Hernandez-Perez & du Toit, 2006), levels of these pathogens in seed lots of varieties used for baby leaf spinach production in the UK have not been established. In addition, the importance of other sources of inoculum for baby leaf spinach production have not been confirmed.

Seed

Objectives

1. To determine the incidence of three fungal pathogens (*S. botryosum, C. dematium* and *C. variabile*) in seed batches of commercial spinach varieties commonly grown in the UK.
2. To determine whether isolates of leaf spot pathogens from spinach seed are pathogenic to spinach.

Methods

For each seed batch tested, four composite samples of 6 g (approximately 500 seeds each) were weighed out. From each of the four 6 g sub-samples, 100 seeds were plated out (400 seeds in total). The mean incidence and standard deviation of seed-borne infection was calculated for each seed batch.

In a preliminary study to test seed plating techniques (Experiment 1), two varieties of spinach seed were supplied by a grower. To remove the fungicide seed treatment, the seed batches were washed repeatedly in distilled water (six 10 minute washes) before proceeding with surface sterilisation and plating out. In a main experiment (Experiment 2), 100 g samples of untreated spinach seed of eight varieties grown in the UK were supplied by one seed company.

For each seed batch, four composite samples of 6 g were weighed out and placed in muslin secured with an elastic band. The seed was surface sterilised in 1.2% sodium hypochlorite for 60 seconds (ensuring that the seed was continuously agitated) then rinsed three times in sterile distilled water (SDW) before drying on sterile paper towel in a laminar flow hood. Filter paper was placed in Petri dishes (one piece per dish) and moistened with 4 ml SDW (20 dishes per seed batch). For each of the four replicate samples, flame-sterilised forceps were used to plate 20 seeds on each of five filter papers. Each Petri dish was sealed with Parafilm. The seeds were incubated in the dark at 24^{o}C for 24 h (to imbibe moisture), then

frozen for 22-24 h at –20°C to prevent further germination. The dishes were placed in a controlled environment cabinet (24°C) for 14 days with a 12 h/12 h light/dark cycle. The seeds were examined at 4 and 12-13 days after plating under a low power microscope. At each assessment, the incidence of *C. variabile*, *S. botryosum*, *C. dematium* and other fungi was recorded.

Colonies identified as *C. variabile*, *S. botryosum* or *C. dematium* during the seed assay were transferred to agar to obtain pure cultures using the agar media described in Section 2.2.

Representative isolates of leaf spot pathogens were used in pathogenicity tests. For each isolate to be tested, three half trays of spinach (var. Lazio) with twelve seeds per tray were sown using M2 compost. When the seedlings had reached the 2 true-leaf stage, they were spray inoculated to run-off with a spore suspension of the fungal isolate (1 x 10^5 spores/ml) in SDW. Three half trays each of twelve spinach seedlings were sprayed with sterile distilled water as uninoculated controls. Each tray was enclosed in a sealable polythene bag and incubated in ambient light at approximately 20°C. The plants were checked for symptom development after 5 and 7 days and typical lesions plated on to PDA+S to confirm the causal organism.

Results and discussion

Colletotrichum dematium was not found in any of the ten seed lots tested from either Experiment 1 or 2 (Tables 2.1 and 2.2). Hernandez-Perez & du Toit (2006) found *Colletotrichum* species in only 3 of 66 seed lots assayed and at very low infection levels (e.g. 0.04% infection in two of 27 seed lots from Denmark in 2003). The nil to low levels of this pathogen being detected in spinach seed lots previously and in this study suggests that other sources of inoculum may be of greater importance in outbreaks of spinach anthracnose in the UK.

Cladosporium variabile was absent from the two seed lots tested in Experiment 1 (Table 2.1) but present at low levels (1.3% or less) in two out of the eight seed batches tested in Experiment 2 (Table 2.2). In a previous study, *Cladosporium* species were recorded in 37 out of 77 seed lots at a mean incidence of 1.8% (Herandez-Perez & du Toit, 2006). In this study, *C. variabile* was distinguished from other *Cladosporium* species (e.g. *C. macrocarpum*) that are non-pathogenic on spinach, by conidial morphology and the presence of spiralling hyphae (Ellis, 1971). Other *Cladosporium* species observed were categorised as ‘other fungi’. The presence of *C. variabile* in spinach seed lots and the

proven transmission of the pathogen from spinach seed to seedlings under glasshouse conditions (Hernandez Perez & du Toit, 2005) suggest that seed-borne inoculum may contribute to outbreaks of Cladosporium leaf spot in the UK. However, given the relatively low frequency of occurrence across seed batches, and low incidence of infection in individual seed batches, other sources of inoculum may also play a role.

Stemphylium botryosum and/or its teleomorph *Pleospora herbarum* were present on all but one of the seed lots in Experiments 1 and 2 (Tables 2.1 and 2.2). *S. botryosum* was identified by the presence of conidia typical of the species. However, the fungus was more commonly present on seed as the teleomorph *P. herbarum*, which was visible as black survival structures (pseudothecia). When plated on to agar media, the pseudothecia gave rise to colonies with mycelium and conidia typical of *S. botryosum.* The combined incidence of *S. botryosum* and *P. herbarum* on individual seed lots ranged from 0.8 to 27.0%. Hernandez & Perez (2006) found *S. botryosum* present in each of 77 spinach seed lots assayed (at a mean incidence of 29% per lot). The high frequency with which seed lots are infected with *S. botryosum* and the proven transmission of the pathogen from spinach seed to seedlings under glasshouse conditions (Hernandez & Perez & du Toit, 2005) suggests that seed-borne inoculum may be of key importance in outbreaks of Stemphylium leaf spot in the UK.

In addition to leaf spot pathogens, *Verticillium dahliae* was detected in eight of the ten seed lots examined in Experiment 2 (Table 2.2). This species was also observed on seed lots assayed in Experiment 1, but was not recorded separately from ‘other fungi’. The fungus was identified on the basis of conidia and conidiophore morphology, and the presence of microsclerotia. On seed lots where it occurred, the incidence of *V. dahliae* varied from 0.3 to 52%. *V. dahliae* is known to be a problem on spinach seed crops in the USA, with both seed and soil acting as possible sources of inoculum (du Toit *et al*., 2005). The development of wilt symptoms due to *V. dahliae* in spinach does not occur until after the initiation of stem elongation and/or flowering (du Toit *et al*., 2005). This explains why *Verticillium* wilt is not a problem in UK production of either processed or baby leaf spinach, despite the use of seed lots with relatively high infection levels.

Alternaria species were found on all seed lots in Experiments 1 and 2, with the incidence of infection exceeding 20% in all but one seed lot. *Alternaria* species are common seed contaminants and are most likely to be saprophytic species, not acting as primary pathogens

of spinach. Other fungal species and bacteria were observed on the spinach seed assayed but not identified further.

Representative isolates of *Stemphylium botryosum*, *Pleospora herbarum*, *Cladosporium variabile* and *Verticillium dahliae* from spinach seed were obtained in pure culture and are currently being tested for their pathogenicity on spinach seedlings. Results will be included in the year 2 Annual Report.

Table 2.1. Incidence of leaf spot pathogens and other micro-organisms on washed fungicide-treated spinach seed of two varieties commonly grown in the UK (Experiment 1)

Variety Code	% seeds affected* (± standard deviation)													
	Stemphylium botryosum		*Pleospora herbarum***		Total *S. botryosum****	*Colletotrichum dematium*		*Cladosporium variabile*		*Alternaria* spp.		Other fungi		Bacteria	
1	1.8	± 3.4	1.0	± 2.1	2.8	0.0	± 0.0	0.0	± 0.0	33.0	± 8.3	7.5	± 7.2	0.5	± 1.5
2	0.0	± 0.0	0.0	± 0.0	0.0	0.0	± 0.0	0.0	± 0.0	2.5	± 3.4	1.3	± 2.2	0.0	± 0.0

* Out of 400
** Teleomorph of *S. botryosum*
*** Total incidence for *S. botryosum* & *P. herbarum*

Table 2.2. Incidence of leaf spot pathogens and other micro-organisms on untreated spinach seed of varieties commonly grown in the UK (Experiment 2)

Variety code	% seeds affected* (± standard deviation)															
	Stemphylium botryosum		*Pleospora herbarum***		Total *S. botryosum****	*Colletotrichum dematium*		*Cladosporium variabile*		*Verticillium dahliae*		*Alternaria* spp.		Other fungi		Bacteria	
1	0.3	± 1.1	13.8	± 9.4	14.1	0.0	± 0.0	1.3	± 2.8	1.0	± 2.1	37.3	± 11.6	24.5	± 15.0	0.0	± 0.0
2	1.0	± 2.1	7.3	± 6.0	8.3	0.0	± 0.0	0.0	± 0.0	9.5	± 8.1	66.8	± 11.3	7.5	± 5.7	0.0	± 0.0
3	1.5	± 2.4	27.0	± 16.3	28.5	0.0	± 0.0	0.0	± 0.0	7.5	± 5.5	66.5	± 12.7	7.5	± 10.9	0.0	± 0.0
4	0.0	± 0.0	0.8	± 1.8	0.8	0.0	± 0.0	0.0	± 0.0	0.3	± 1.1	47.0	± 13.2	18.3	± 9.9	1.5	± 3.7
5	0.5	± 1.5	14.3	± 9.8	14.8	0.0	± 0.0	0.3	± 1.1	0.3	± 1.1	50.5	± 10.8	9.0	± 7.9	0.0	± 0.0
6	0.5	± 1.5	16.8	± 9.9	17.3	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	32.8	± 11.4	24.8	± 11.5	0.0	± 0.0
7	0.0	± 0.0	2.8	± 3.8	2.8	0.0	± 0.0	0.0	± 0.0	51.8	± 10.7	23.8	± 10.8	8.3	± 6.7	0.0	± 0.0
8	0.8	± 1.8	17.5	± 11.1	18.3	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	87.5	± 8.8	3.5	± 4.3	0.0	± 0.0

* Out of 400
** Teleomorph of *S. botryosum*
*** Total incidence for *S. botryosum* & *P. herbarum*

Volunteer plants

Introduction and objective

A sample of spinach plants (var. Fiorano) was sent from a commercial holding in Hampshire in February 2007. The grower reported that the plants originated from a part of a field where crop destruction by herbicide at the end of the previous autumn (2006) had been incomplete. The plants had re-sprouted and showed abundant leaf spotting. There was concern that the volunteer plants could act as a source of inoculum for disease on new crops planted in February.

The aim of the study was to investigate the cause of leaf spotting on the volunteer spinach. In addition, the infected plant debris has been stored in soil and will be used in a further study to determine whether disease transmission can occur from infested soil to spinach seedlings.

Methods

Leaf lesions were examined microscopically. Leaf tissue from the edge of the lesions was surface sterilised in 1% sodium hypochlorite (15 seconds), rinsed in SDW, dried on filter paper then plated on PDA+S. A fungus typical of *Colletotrichum dematium* was consistently isolated.

An isolate of *C. dematium ex* volunteer spinach (AR 07/09) was used in pathogenicity tests. Six half trays of spinach (var. Lazio) with twelve seeds per tray were sown using M2 compost. When the seedlings had reached the 2 true-leaf stage, they were spray-inoculated to run-off with a spore suspension of the fungal isolate (1 x 10^6 spores/ml) in SDW. Control plants were sprayed with sterile distilled water as uninoculated controls. Each tray was enclosed in a sealable polythene bag and incubated in a controlled environment cabinet at approximately 20^{o}C. The plants were checked for symptom development after 3 and 7 days and typical lesions examined microscopically and plated on to PDA+S to confirm the causal organism.

Results and discussion

The leaf lesions were pale, and variable in size. Setae and sporulation typical of *Colletotrichum dematium* was visible microscopically, and typical cultures of *C. dematium* developed when affected leaf tissue was plated out.

In the pathogenicity test, anthracnose lesions developed within 3-4 days on inoculated plants and the plants died rapidly. *C. dematium* was confirmed as the causal organism by microscopic examination of lesions and by culturing of affected leaf tissue. Uninoculated plants remained healthy.

The study confirms that *C. dematium*, causal organism of anthracnose on spinach, can overwinter on volunteer spinach in the UK. Viable spores of the pathogen were present within leaf lesions on the volunteer plants. Spores of *Colletotrichum* species are readily dispersed by rain splash and could pose a risk to emerging spinach crops in spring. Further studies will determine whether weeds or other crop species are hosts to *Colletotrichum dematium ex* spinach.

Fungicide efficacy

Objectives

- To determine the efficacy of fungicides applied at different timings in relation to infection, for control of spinach leaf spots caused by *Colletotrichum dematium*, *Cladosporium variabile* and *Stemphylium botryosum*
- To determine the crop safety of the products used.

Methods

The treatments shown in Table 2.3 were tested in separate experiments against spinach anthracnose caused by *C. dematium* (Experiment 1), Cladosporium leaf spot caused by *C. variabile* (Experiment 2) and Stemphylium leaf spot caused by *S. botryosum* (Experiment 3).

Table 2.3. Fungicide/product treatments evaluated for control of spinach leaf spots

	Fungicide/product	Time of application with respect to artificial inoculation (days)
1	Untreated control	Nil
2	Untreated control	Nil
3	Untreated control	Nil
4	Amistar	-3
5	Amistar	-1
6	Amistar	+1
7	Signum	-3
8	Signum	-1
9	Signum	+1
10	Teldor	-3
11	Teldor	-1
12	Teldor	+1
13	Switch	-3
14	Switch	-1
15	Switch	+1
16	Plover	-3
17	Plover	-1
18	Plover	+1
19	Folicur	-3
20	Folicur	-1
21	Folicur	+1
22	Pre-Tect	-3
23	Pre-Tect	-1
24	Pre-Tect	+1

Each experiment comprised a two-way factorial design with ten plants per plot and four replicate blocks. There were seven fungicide/product treatments applied at three different timings, with a full replication of the inoculated untreated control for each timing, to give a total of 24 treatments and 96 plots. A plot comprised a tray of ten spinach plants, artificially inoculated with *C. dematium* (Experiment 1), *C. variabile* (Experiment 2) and *S. botryosum* (Experiment 3). Four extra trays of 10 seedlings were placed away from the main trial area,

to avoid infection via spore splash, as uninoculated untreated controls (not included in statistical analyses). Data for disease severity (percentage plant area affected by symptoms) was analysed by ANOVA.

For each of Experiments 1, 2 and 3, spinach seeds were sown in F1 compost in ½ size seed trays, with 12 seeds per tray thinned to ten seeds (2 rows of 5) prior to spray applications. The trays were laid out in four blocks (two rows per block). For Experiments 1 and 2 done in the summer, the trays were placed on a hard standing area (within rabbit fencing), raised off the ground and covered with crop mesh to prevent pigeon and insect damage. For Experiment 3 done in the spring, seedlings were raised in a heated glasshouse. The trays were moved outside for treatment applications, and then transferred to a polytunnel for subsequent maintenance and assessments. In all experiments, the uninoculated control trays were placed at least 5 m from the main trial area. All trays were overhead watered as required to maintain moist but not water-logged compost. The trays were maintained until plants reached the 2 true-leaf stage (14-21 days after planting).

For Experiment 1, an isolate of *C. dematium ex* spinach (AR 05/189) was sub-cultured onto 50 plates of ¼ strength PDA+S about 14 days before required for inoculation. The plates were incubated in the dark for approximately 3 days at 20°C, and then transferred to UV light (about 18°C) to encourage sporulation. On the day of inoculation, the plates were used to prepare a spore suspension of *C. dematium*. A sterile spatula was used to scrape spores into distilled water. The spore suspension was filtered through muslin and adjusted to a concentration of 7.7×10^5 spores/ml. The four uninoculated control trays were sprayed with distilled water using a hand-held mister. Subsequently, each of 96 trays on the hard standing area was sprayed to the point of run-off using the spore suspension of *C. dematium*. Immediately after inoculation (done late afternoon), the trial area was covered with a 'tent' of polythene sheeting (avoiding contact with plants) that was kept over the trays for approximately 16 h to prolong leaf wetness duration and to promote high relative humidity.

A similar method was used in Experiment 2 to inoculate plants with *C. variabile* except the fungal isolate (code AR 06/74a) was grown on PDA+S in the dark. A spore concentration of 1.3×10^5 spores/ml was used for inoculation.

A similar method was used in Experiment 3 to inoculate plants with *S. botryosum*. An isolate of *S. botryosum ex* spinach seed was used (isolate code: AR 07/14). The fungus was cultured on V8 agar and incubated at 20°C, 12 h light/12 h dark. A spore concentration of 1.2×10^5 spores/ml was used for inoculation.

For Experiments 1, 2 and 3, treatments were applied either 3 days before, 1 day before, or 1 day after artificial inoculation (according to the treatment list in Table 2.3). Products were applied in 1000 L water/ha (100 ml/m^2) using an Oxford precision sprayer with single nozzle (plus guard to prevent spray drift) at 2 Bar pressure. The crop mesh was removed prior to product application. Product rates are shown in Table 2.4.

Table 2.4. Product rates applied to spinach to evaluate efficacy against leaf spot diseases

Product	Active ingredient	Product rate
Amistar	Azoxystrobin	1 L/ha
Signum	Boscalid + pyraclostrobin	1.5 kg/ha
Teldor	Fenhexamid	1.5 kg/ha
Switch	Cyprodonil + Fludioxonil	1 kg/ha
Plover	Difenoconazole	0.5 L/ha
Folicur	Tebuconazole	0.75 L/ha
Pre-Tect	-	1 kg/ha

Notes:

Amistar	Previously LTAEU from outdoor lettuce (SOLA 1465/01); LTAEU now expired
Signum	SOLA 2378/05
Teldor	SOLA 0026/05
Switch	Administrative Experimental Approval
Plover	Administrative Experimental Approval (use rate from SOLA 0558/05 on pak choi)
Folicur	Administrative Experimental Approval (use rate from SOLA 1876/03 on baby leaf brassicas)
Pre-Tect	Not marketed as a fungicide product. Contains the bacterial protein Harpin, reported to trigger plant biochemical pathways that stimulate certain growth and stress-defence responses

In each experiment, the plants were monitored regularly (approximately every 3 days) for symptom development after the 3rd fungicide application. For each tray, the incidence and severity of leaf spot symptoms were recorded. Phytotoxic symptoms or growth benefits were described and the proportion of plants affected was recorded.

In each experiment, typical leaf spot symptoms were examined microscopically, and subsequently plated on PDA+S (after surface sterilising in 1% sodium hypochlorite for 30 sec, followed by a rinse in sterile distilled water) and incubated at 20°C, to confirm the causal organism.

Results and discussion

Spinach anthracnose (Experiment 1)

anthracnose lesions were first visible 7 days after inoculation and disease severity was assessed 13 days after inoculation. Since lesions were very small (1-2 mm diameter), the severity of disease was assessed by counting the number of lesions per plant rather than by estimating percentage area affected. Later assessments were not done because rapid secondary disease spread could have confused treatment effects. *C. dematium* was confirmed as the causal organism by microscopic examination and culturing. Uninoculated control plants remained symptom free.

There was a significant effect of fungicide timing on disease development, with better disease control obtained when sprays were applied either 1 day before or 1 day after inoculation compared with 3 days before inoculation (Table 2.5). When disease severity was averaged across the three product timings, all of the products apart from Teldor significantly reduced disease severity. Amistar and Signum provided excellent disease control (mean of 0.5 lesions per plant or less), irrespective of product timing. Mean lesion number per plant was also reduced to 0.5 or less when Switch or Pre-Tect were applied 1 day before or after inoculation, and when Folicur was applied 1 day after inoculation.

Table 2.5. Effect of product application at different timings on severity of spinach anthracnose (*Colletotrichum dematium*), 13 days after inoculation

Product	Mean no. lesions per plant after different product application timings			Product means
	T1*	T2	T3	
Untreated control	7.1	2.7	3.6	4.4
Amistar	0.1	0.0	0.5	0.2
Signum	0.0	0.4	0.1	0.2
Teldor	5.3	0.9	1.5	2.6
Switch	1.8	0.3	0.5	0.8
Plover	3.6	1.1	2.3	2.3
Folicur	1.6	1.9	0.4	1.3
Pre-Tect	2.3	0.2	0.3	1.0
Timing means	2.7	0.9	1.1	1.6
		L.s.d = 1.19 D.f. = 69 *P*<0.001		L.s.d. = 1.94 D.f. = 69 *P*=0.007

*Fungicides applied at –3 days (T1), -1 days (T2) and +1 days (T3) with respect to artificial inoculation.

All plants treated with Amistar 1 day before inoculation developed symptoms of phytotoxicity ('scorch' lesions) within 1 day of treatment. Subsequent Amistar applications were not observed to by phytotoxic. None of the other products caused symptoms of phytotoxicity. Plants in plots treated with Signum were observed to be darker green in colour than those in untreated control plots.

Cladosporium leaf spot (Experiment 2)

Leaf lesions were first visible 7 days after inoculation and disease severity was assessed 13 days after inoculation. The severity of disease was assessed by estimating the percentage leaf area affected per plant. Later assessments were not done because rapid secondary disease spread could have confused treatment effects. *C. variabile* was confirmed as the causal organism by microscopic examination and culturing. Uninoculated control plants remained symptom free.

Disease severity exceeded 50% leaf area affected in the untreated control plots. All of the products significantly reduced disease severity (Table 2.6), but disease levels remained unacceptably high (>15%) in plots treated with Teldor, Switch and Pre-Tect. Signum and Plover proved excellent control of Cladosporium leaf spot irrespective of product timing. Amistar and Folicur gave moderate leaf spot control, with Amistar being most effective when applied 1 day either side of inoculation. There was a significant effect of product timing, with lower disease levels overall when products were applied 1 day after inoculation.

Table 2.6. Effect of product application at different timings on severity of Cladosporium leaf spot (*Cladosporium variabile*), 13 days after inoculation

Product	% plant area affected after different product application timings			Product means
	T1*	T2	T3	
Untreated control	49.3	57.0	49.5	51.9
Amistar	6.4	1.5	2.0	3.3
Signum	1.0	0.0	0.7	0.6
Teldor	22.3	29.3	16.3	22.6
Switch	17.8	15.3	18.8	17.3
Plover	1.0	0.5	1.5	1.0
Folicur	11.8	8.4	6.6	8.9
Pre-Tect	36.0	31.8	20.1	29.3
Timing means	18.2	18.0	14.4	16.8
		L.s.d. = 3.29		L.s.d. = 5.37
		D.f = 69		D.f. = 69
		P=0.044		*P*<0.001

*Fungicides applied at –3 days (T1), -1 days (T2) and +1 days (T3) with respect to artificial inoculation.

No symptoms of phytotoxicity or beneficial growth effects were observed in this experiment.

Experiment 3

There was no symptom development due to inoculation with *S. botryosum* in Experiment 3. This may have been due to low pathogenicity of the isolate used for inoculation (to be confirmed) or due to sub-optimal conditions for plant inoculation. This experiment will be repeated. Symptoms of phytotoxicity ('scorching') were observed on plants treated with Amistar, following the second fungicide application. No other phytotoxic or beneficial effects were observed.

Summary

Of the products tested, Signum provided the most consistent control of two leaf spot pathogens, *Colletotrichum dematium* and *Cladosporium variabile*, with opportunity for disease control when applied up to 3 days before or 1 day after an infection event. Of other products currently approved for spinach, Teldor did not provide adequate disease control. Of products not currently permitted on spinach or not marketed as fungicides, Amistar was effective for disease control but occasionally phytotoxic (in agreement with grower observations). Switch, Folicur, Plover and Pre-Tect each provided excellent control of one pathogen but not both.

Varietal susceptibility to leaf spot pathogens

Objective

To compare 12 spinach varieties used by commercial growers for their susceptibility to leaf spot pathogens, *Colletotrichum dematium* and *Cladosporium variabile*

Methods

Twelve spinach varieties (provided as seed by a commercial grower) were tested for susceptibility to each of the two leaf spot pathogens, *C. dematium* (Experiment 1) and *C. variabile* (Experiment 2). The spinach varieties used are listed in Table 2.7.

Each experiment comprised a two-way factorial design with four plants per plot and four replicate blocks. Twelve varieties were evaluated, each either inoculated or as uninoculated controls. There were four plants per plot and four replicate blocks (96 pots in total). A plot comprised a 9 cm pot containing four spinach plants. Two blocks (48 pots) were located in each of two controlled environment cabinets, running with the same temperature, relative humidity (RH) and lighting conditions. Two runs of the experiment were done for each pathogen. Data for disease severity (percentage plant area affected by symptoms) was analysed by ANOVA.

For each run of the experiment, spinach seeds were sown in 9 cm diameter pots in F1 or M2 compost, with 5 seeds per pot and eight pots per variety. During the summer, the pots were placed in a well-ventilated polytunnel until emergence then transferred to a hardstanding area under crop mesh. During the autumn and winter, plants were raised in a heated glasshouse. The plants were overhead watered as required to maintain moist but not water-logged compost. The pots were maintained until plants reached the 2 true-leaf stage then where needed, the plants were thinned to four seedlings per pot.

To test spinach varietal susceptibility to anthracnose (Experiment 1), an isolate of *C. dematium ex* spinach (isolate code AR 05/189, known to be pathogenic to spinach) was sub-cultured onto 10 plates of ¼ strength PDA+S at least 14 days before required for inoculation. The plates were incubated for approximately 3 days at 20^{o}C, then transferred to UV light (approximately 18^{o}C) to encourage sporulation. On the day of inoculation, a sterile loop was used to scrape spores from the cultures of *C. dematium* into distilled water. The spore suspension was filtered through muslin and adjusted to a concentration of 1 x 10^{6} spores/ml. Pots for the uninoculated control treatments (48 in total) were sprayed with distilled water

using a hand-held mister, and placed individually in sealable polythene bags. Subsequently, each of the 48 remaining pots were sprayed to the point of run-off using the spore suspension of *C. dematium,* and placed individually in sealable polythene bags. The pots were placed in controlled environment cabinets set at 20°C, 40% RH and 16 h light/8 h dark.

The plants were assessed after 3 and 7 days with the bags left unsealed after the first assessment. For each plant, the incidence and severity of leaf spot symptoms were recorded. Typical leaf lesions were plated on PDA+S (after surface sterilising in 1% sodium hypochlorite for 15 seconds, followed by a rinse in sterile distilled water) and incubated at 20°C to confirm the causal organism.

A similar method and experimental design was used to test spinach varietal susceptibility to Cladosporium leaf spot. An isolate of *Cladosporium variabile* known to be pathogenic to spinach (isolate code: AR06/74a) was cultured on PDA+S incubated in the dark at 20°C. A spore concentration of 1 x 10^5 spores/ml was used for inoculation.

A similar method will be used to test varietal susceptibility to *Stemphylium botryosum* once pathogenicity of a suitable isolate has been confirmed.

Results and discussion

The experiments were done under high inoculum pressure to ensure disease development. In all experiments, the uninoculated plants remained symptom-free, indicating that disease development was as a result of artificial inoculation, rather than seed-borne inoculum.

Colletotrichum dematium

Under conditions of high inoculum pressure and favourable environmental conditions, none of the varieties remained symptom-free. *C. dematium* was confirmed as the cause of lesion development on inoculated plants by microscopic examination and culturing. There was no effect of variety on disease incidence, with 100% incidence of anthracnose on inoculated plants in both runs of the experiment, at 7 days after inoculation. In run 1, there were significant differences in disease severity (P<0.001) between the varieties but these differences were less marked (not significant) in run 2 (Figure 2.1). Matisse and RZ 51-309 were the least susceptible varieties in run 1 with percentage leaf area affected at least half of that recorded on other varieties. Matisse also ranked as the least susceptible variety in run 2, followed by Monza (Table 2.7). Lazio and Emelia were two of the most susceptible varieties in both runs of the experiment (Figure 2.1, Table 2.7).

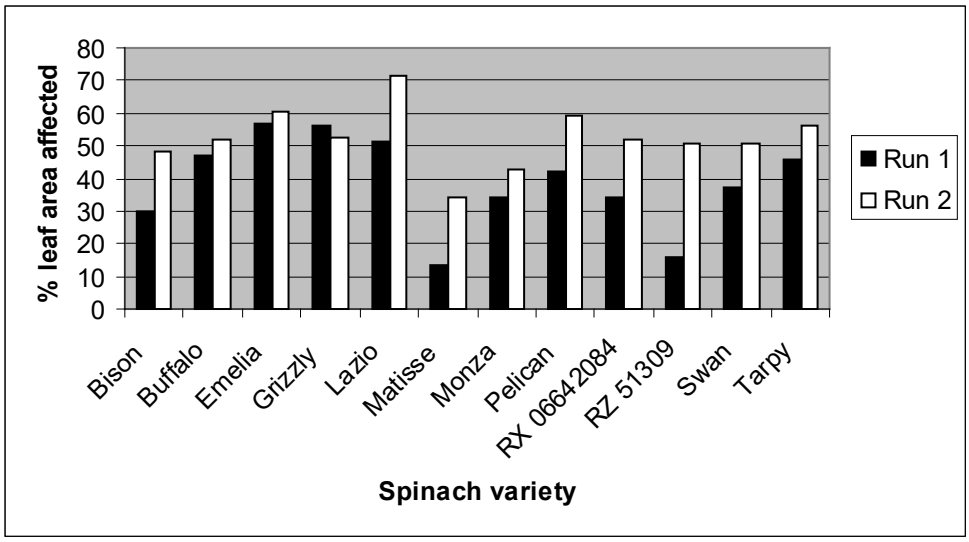

Figure 2.1. Susceptibility of 12 spinach varieties to *Colletotrichum dematium*, 7 days after inoculation

Cladosporium variabile

Under conditions of high inoculum pressure and favourable environmental conditions, none of the varieties remained symptom-free. *C. variabile* was confirmed as the cause of lesion development on inoculated plants by microscopic examination and culturing. There was no effect of variety on disease incidence, with 100% incidence of anthracnose on inoculated plants in both runs of the experiment, at 7 days after inoculation. In run 2, there were significant differences in disease severity (P<0.001) between the varieties but these differences were less marked (not significant) in run 1 (Figure 2.2). RZ 51-309, Monza and Tarpy were the three least susceptible varieties in both runs (Table 2.7). Percentage leaf area affected for these varieties was 11% or less in both runs, compared with 36% in the worst affected variety (Lazio). Lazio and Buffalo were two of the most susceptible varieties in both runs of the experiment (Figure 2.1, Table 2.7).

In summary, none of the spinach varieties tested were resistant to the leaf spot pathogens *C. dematium* (spinach anthracnose) or *C. variabile* (Cladosporium leaf spot). However, for some varieties, there was consistency in their level of susceptibility to one or both pathogens (Table 2.6). Varieties that showed lower susceptibility to a single pathogen were Matisse (versus *C. dematium*) and Tarpy (versus *C. variabile*). RZ 51-309 and Monza appeared most promising as varieties with lower susceptibility to both pathogens. Lazio was highly susceptible to both spinach anthracnose and Cladosporium leaf spot, and this finding was in agreement with grower observations.

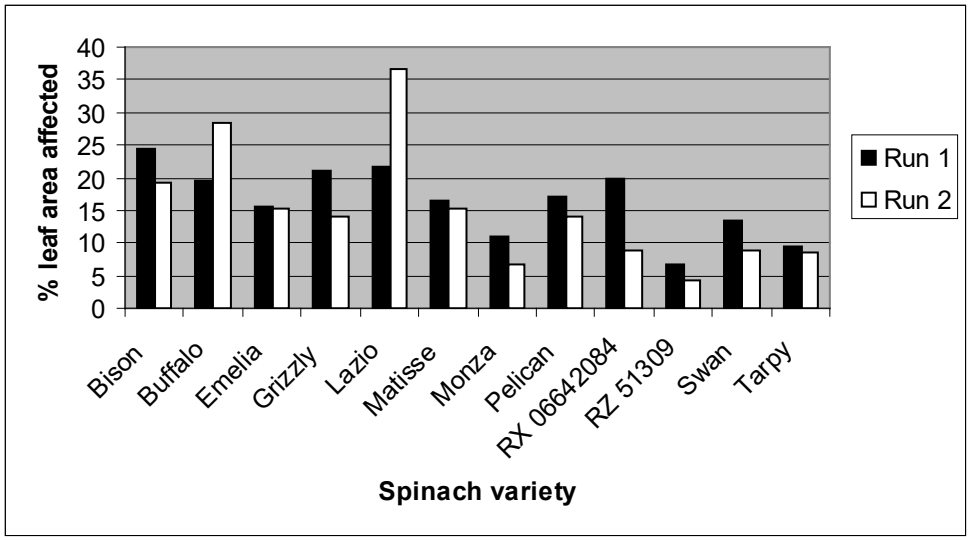

Figure 2.2. Susceptibility of 12 spinach varieties to *Cladosporium variabile*, 7 days after inoculation

Table 2.7. Ranked susceptibility of 12 spinach varieties to two leaf spot pathogens, following artificial inoculation using spore suspension

	Variety	Ranking in susceptibility to *C. dematium**		Ranking in susceptibility to *C. variabile**		Overall ranking
		Run 1	Run 2	Run 1	Run 2	
1	Lazio	10	12	11	12	12
2	Monza	=4	2	3	2	2
3	Matisse	1	1	6	8	3
4	Swan	6	4	4	=4	4
5	Pelican	7	10	7	=6	8
6	Bison	3	3	12	10	7
7	Buffalo	9	=6	8	11	9
8	Emelia	12	11	5	=8	11
9	Tarpy	8	9	2	3	5
10	Grizzly	11	8	10	=6	10
11	RX 06642084	=4	=6	9	=4	6
12	RZ 51-309	2	5	1	1	1

*1=least susceptible, 12 = most susceptible

Conclusions

Sources of inoculum

Seed

Stemphylium botryosum (Stemphylium leaf spot) and *Cladosporium variabile* (Cladosporium leaf spot) were detected in seed lots of spinach varieties used for UK baby leaf production. *S. botryosum* was most prevalent, being found in 9 out of 10 seed lots, with percentage incidence ranging from 0.8 to 27%. *Colletotrichum dematium* (spinach anthracnose) was not detected in any of the seed lots suggesting that other sources of inoculum are responsible for outbreaks of this disease in the UK. *Verticillium dahliae* was also detected six out of eight spinach seed lots tested. Although this pathogen does not affect spinach grown for fresh and processing use, its presence on seed is of concern since it may affect other crops in the rotation, emphasising the need for an effective seed treatment.

Volunteer plants

Colletotrichum dematium (producing viable conidia) was confirmed on overwintering spinach volunteers at a commercial holding, demonstrating a potential source of inoculum for spinach anthracnose.

Fungicide efficacy

Of seven products tested in inoculated pot trials, Signum (boscalid + pyraclostrobin) provided the most consistent control of two leaf spot pathogens, *Colletotrichum dematium* and *Cladosporium variabile*, with opportunity for disease control when applied up to 3 days before, or 1 day after an infection event. Of other products currently approved for spinach, Teldor (fenhexamid) did not provide adequate control of these two diseases. Of products not currently permitted on spinach or not marketed as fungicides, Amistar (azoxystrobin) was effective for disease control but occasionally phytotoxic (in agreement with grower observations). Switch (cyprodonil + fludioxonil), Folicur (tebuconazole), Plover (difenoconazole) and Pre-Tect (Harpin) each provided excellent control of one pathogen but not both.

Varietal susceptibility

In artificially inoculated pot experiments, none of 12 spinach varieties tested were resistant to the leaf spot pathogens *C. dematium* (spinach anthracnose) or *C. variabile* (Cladosporium leaf spot). However, for some varieties, there was consistency in their level of susceptibility to one or both pathogens. RZ 51-309 and Monza appeared most promising as varieties with lower susceptibility to both pathogens. Lazio was highly susceptible to both spinach

anthracnose and Cladosporium leaf spot, and this finding was in agreement with grower observations.

Technology transfer

- Telephone and email responses to growers requesting information on sample diagnosis in 2006-2007.
- Green KR. 2006. Spinach leaf spots and their management. HDC Factsheet 08/06. East Malling Kent: Horticultural Development Council. 8 pp.
- Green KR. 2006. Spotter's guide to spinach diseases. *HDC News*, September 2006, pp 26-27.

References

Du Toit LJ, Derie ML & Hernandez-Perez P. 2005. *Verticillium* wilt in spinach seed production. *Plant Disease* **89**: 4-11.

Ellis MB. 1971. *Cladosporium*. Page 315, In: *Dematiaceous Hyphomycetes*. Surrey, UK: Commonwealth Mycological Institute.

Hernandez-Perez P & du Toit LJ. 2005. Prevalence of *Stemphylium botryosum* and *Cladosporium variabile* in commercial spinach seed, and their potential for seed transmission. *Phytopathology* **95**:S41 (abstract).

Hernandez-Perez P & du Toit LJ. 2006. Seedborne *Cladosporium variabile* and *Stemphylium botryosum* in spinach. *Plant Disease* **90**: 137-145.

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