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Horticultural Development Council

Biology and integrated management of leaf spot on spinach and chard - phase I

FV 268

May 2006

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The results and conclusions in this report are based on a series of experiments conducted over one year. 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.

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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.

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1 GROWER SUMMARY

1.1 Headline

- Leaf spots on baby leaf spinach in the UK were caused by infection with *Stemphylium botryosum*, *Colletotrichum dematium* and *Cladosporium variabile*.
- These three pathogens are known to be seed-borne.
- *Stemphylium botryosum* has not previously been reported as a pathogen of spinach in the UK.

1.2 Background and expected deliverables

Major UK producers 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. Spinach leaf spots can be caused by a range of pathogens and the relative importance of different pathogens may vary between farms. Leaf spots can also be problematic on chard crops.

The overall aim of the project is to reduce losses due to leaf spot on spinach and chard through improved knowledge of the biology and control of key pathogens.

The specific objectives of Phase I of the project were:

- To determine the identity of pathogens causing leaf spot diseases of spinach and chard in the UK.
- To conduct a review of current knowledge on the biology and management of leaf spot diseases of spinach and chard and to identify knowledge gaps.
- To produce a Factsheet to incorporate current knowledge on disease identification, biology, and control (chemical and cultural) for leaf spot pathogens on spinach and chard.

1.3 Summary of the project and main conclusions

1.3.1 Leaf spot diseases of spinach and chard

- Samples of baby leaf spinach were received from the UK in 2005-2006 (12 samples) and from Spain in winter 2004/2005 and autumn 2005 (35 samples).
- Lesions on UK samples were caused by infection with the fungal organisms - *Stemphylium botryosum*, *Colletotrichum dematium* and *Cladosporium variabile.*
- *S. botryosum* in known to be a causal agent of spinach leaf spot in the USA but has not previously been recorded as a pathogen of spinach in the UK.

Following pathogen identification by Central Science Laboratory, the Plant Health and Seeds Inspectorate was informed.

- Lesions on Spanish samples were most frequently caused by infection with *Stemphylium botryosum*. *Cercospora* sp. was identified on one sample. Secondary infection due to *Alternaria* spp. and *Fusarium* spp. had frequently occurred on deteriorated leaf tissue.
- Leaf spots caused by different fungi were similar and were difficult to diagnose by visual examination only. Because of the fragile nature of baby leaf spinach, leaves with lesions were prone to secondary infection and general leaf deterioration quickly occurred. Some Stemphylium leaf spots could be confused with pesticide scorch symptoms.
- Typical symptoms due to the most commonly encountered pathogens are shown in Table 1.
- Stemphylium leaf spots were typically pale in colour and circular, with a green watersoaked border. Fungal structures were often absent except in older lesions. Anthracnose leafspots (*Colletotrichum dematium*) were initially seen as indistinct pale yellow/green lesions that later became more defined and turned light brown in colour, either circular or oval. Spore masses (acervuli) were visible within lesions under low power magnification. *Cladosporium* lesions were pale in colour with a well-defined margin. Olive-green sporulation was visible on older lesions under low power magnification.
- Samples of chard were received from Spain (2 samples) and the UK (3 samples). All but one chard sample had purple pin-prick lesions. No fungal structures were associated with the lesions and no pathogens were isolated. Because of the limited number of samples received, it was not possible to determine the main pathogens causing disease problems on chard in the UK.

1.3.2 Knowledge review

Growers and consultants confirmed that up until three years ago in the UK, downy mildew was the only disease problem encountered on baby leaf spinach. Since then, leaf spots have become an increasing problem. Similarly in Spain, leaf spot diseases occur with increasing frequency as growers are steadily expanding production and suitable land is being over-cropped. Growers observed that certain varieties are more affected than others and this may be due either to greater disease susceptibility or to the use of infected seed.

A knowledge review completed in phase I of the project confirmed the following for the three leaf spot pathogens encountered on UK spinach crops:

- *S. botryosum*, *C. variabile* and *C. dematium* can all survive on seed. Transmission from seed to emerging plants has been demonstrated experimentally for *S. botryosum* and *C. variabile* but not for *C. dematium*.

- Seed treatment methods (hot water treatment and disinfectants) for controlling seed-borne diseases of spinach have been investigated and shown to be partially effective.
- Other sources of inoculum for the diseases on spinach in the USA include woody crop debris (for *S. botryosum* and *C. dematium*) and volunteer plants (*C. variabile*).
- Isolates of *S. botryosum* from spinach were only pathogenic to spinach and did not infect other hosts (including closely-related crop and weed hosts), although it is also the species is also a common saprophyte. *C. variabile* and *C. dematium* from spinach were slightly pathogenic on beet in laboratory tests but cross-infection in the field has not been demonstrated.
- Environmental conditions: infection by *S. botryosum* may take place over a wide range of conditions but is favoured by temperatures in the range 18-24°C and prolonged leaf wetness periods. For *C. variabile*, a temperature range of 15-20°C with relative humidity above 80% is most conducive to disease development. Conditions favouring infection by *Colletotrichum* species include high relative humidity and leaf wetness.
- Little is known about the variation in resistance of commercial spinach cultivars to *S. botryosum*, *C. dematium* or *C. variabile.*
- On spinach seed crops in Washington State (USA), fungicides with the following active ingredients gave the most effective control of Stemphylium and *Cladosporium* leaf spots: azoxystrobin, pyraclostrobin, pyraclostrobin + boscalid, iprodione, and kresoxim-methyl, reducing the severity of leaf spots from 19% to 2% or less (du Toit *et al.*, 2003). Fungicide activity against spinach anthracnose has not been reported.

1.3.3 Gaps in knowledge

Gaps in knowledge on the biology and management of spinach leaf spot in the UK were identified as follows:

- The seed-borne nature of *S. botryosum*, *C. dematium* and *C. variabile* has been reported in scientific literature. However, the presence of these pathogens in seed lots of varieties widely used for baby leaf spinach production in the UK has 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 and *Cladosporium* leaf spot symptoms were observed on spinach volunteers. However, the role of infected crop debris in the development of leaf spot diseases in intensive baby-leaf spinach production under UK conditions needs to be ascertained.

- It is not known whether the risk of leaf spot diseases developing is increased by re-use of crop meshes on subsequent crops. This topic is the subject of a separate HDC project (FV 283).
- Lesions due to 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 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, such as Lazio, Whale, Falcon, Tarpy and Compania.
- There is currently little scope for chemical control of spinach leaf spot diseases because of limited product approvals and MRLs. Fungicides approved for control of downy mildew in the UK (fosetyl-aluminium and metalaxyl-M) are generally ineffective against leaf spot pathogens. A protectant fungicide such as Amistar (azoxystrobin) could minimise onset of infection, but has limited eradicant activity and there are reports of phytotoxicity. There may be potential for disease control using fungicides such as Signum (boscalid + pyraclostrobin) which gave promising results on seed crops in the USA, or new products such as Switch (cyprodinil + fludioxonil) (not currently approved for use on spinach). The efficacy of a range of fungicides for management of spinach leaf spots will be evaluated in Phase II of the project.
- 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.

Table 1. Comparison of three fungal leaf spot diseases of spinach

	Stemphylium leaf spot	**Anthracnose**	***Cladosporium* leaf spot**
Pathogen name	*Stemphylium botryosum*	*Colletotrichum dematium*	*Cladosporium variabile*
Symptoms	Tan lesion with green watersoaked margin	Initially, pale yellow irregular lesions, then light brown	Pale lesions

		and shot-holed	
			
Fungal structures in leaf spots	Only in old lesions	Yes	Olive-green sporulation on older lesions
Seedborne	Yes	Yes	Yes
Dispersal	Wind, seed	Water splash, seed	Wind, seed
Overwintering	Woody spinach debris, seed	Volunteers, seed	Volunteers, seed
Favourable conditions	Moist, warm	Wet, cool	Moist, cool
Host range	Spinach only	Chenopod species	Chenopod species

Source: http://mtvernon.wsu.edu/path_team/currentnewlet.htm

Table 2. Pathogenicity of fungal leaf spot pathogens on crop hosts of the Chenopodiacae family

Pathogen	**Crop host**			
	Spinach (*Spinacea oleracea*)	Red chard (*Beta vulgaris*)	Spinach beet (*Beta vulgaris*)	Sugar beet (*Beta vulgaris*)
Stemphylium botryosum	✓	X	X	X*
Cladosporium variabile	✓	?	?	?
Colletotrichumdematium	✓	?	?**	?
Cercospora beticola	✓	✓	✓	✓
Ramularia beticola	✓	✓	✓	✓
Phoma betae	✓	✓	✓	✓

*can be a common saprophyte on dead or damaged plant material

** reported in Defra DOVE project in an organic spinach beet crop

1.4 Financial benefits

Spinach and chard producers 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 in 2003.

It is intended that the industry will benefit through reduced losses due to spinach and chard leaf spot, achieved in Phase I of the project through increased knowledge of the identify and biology of pathogens causing leaf spot. Phase II commencing in April 2006 focuses on disease management.

1.5 Action points for growers

- Ensure correct diagnosis of leaf spot pathogens. Correct diagnoses are important because they can aid correct fungicide selection, improve the timing of fungicide applications, assist with the selection of higher levels of resistance and determine appropriate cultural practices for more effective management.
- Key leaf spot pathogens of spinach can be seed-borne and routine seed health testing methods are available. Check the health status of seed before use.
- Ensure fields are free of volunteers.
- Bury, burn or dispose carefully of crop debris that could potentially be a source of inoculum for leaf spot diseases.

2 SCIENCE SECTION

2.1 Introduction

With multiples allocating increasing shelf space to prepared salads, there is a continuing increase in spinach production in the UK and world-wide. One major grower reports a 20% increase in spinach production for each of the last three years

Major UK producers report that leaf spot diseases are becoming an increasing constraint to the production of baby- and mid-leaf spinach crops and chard, for which there is zero tolerance of leaf blemishes. Although leaf spots can occur at any time during the growing season, they tend to be more prevalent on late summer/autumn crops, which have a longer growing period, and a higher risk of wet conditions conducive for pathogen development. Leaf spot diseases are also an increasing constraint to cropping overseas (Spain and Portugal), where production is steadily expanding and suitable land is intensively cropped.

Spinach leaf spots can be caused by a range of pathogens and the relative importance of different pathogens may vary between farms. For example, one producer has reported crop losses due to *Cladosporium variabile*, while another reported leaf spots due to *Colletotrichum* sp. Leaf spot in chard is thought to be caused by other pathogens.

Potential sources of inoculum for the pathogens include seed, crop debris and alternative hosts. Testing to date has not identified seed as a major source of inoculum for *C. variabile* in the UK, and limited information is available for other pathogens. Survival on crop debris could be of particular relevance when repeated cropping occurs in the same field. In addition, there is little information available on the potential for leaf spot pathogens of sugar beet, chard and related chenopod weeds (e.g. *Ramularia*, *Cercospora* and *Phoma*), to infect spinach.

The objective of Phase I of this project was to confirm the primary causal pathogens of spinach leaf spot in the UK by identifying pathogens present on samples submitted by growers. A knowledge review was done to compile currently available information on disease biology and management options, as the basis for a grower factsheet.

2.2 Pathogen identity

2.2.1 Methods

UK growers of baby leaf spinach and chard were requested to send samples of spinach with typical leaf spot symptoms. Where possible, growers wrapped individual dry leaves in newspaper or paper towel prior to postage. Samples received were photographed and symptoms described. Leaf lesions were examined microscopically before and after damp incubation (leaves placed on paper towel moistened with distilled water in plastic boxes with a lid and incubated at approximately 20°C in ambient light). To determine the causal pathogens, tissue pieces (3 mm^2) cut from the leading edge of leaf lesions was surface sterilised (30 sec in 1% sodium hypochlorite and 1 min wash in distilled water) or plated directly onto potato dextrose agar amended with streptomycin (PDA+S), and incubated at 20°C in the dark. Once pure cultures of isolated fungi had been obtained, they were sub-cultured onto slopes of PDA+S and maintained in a refrigerator. Other agar media were used where necessary to induce fungal sporulation (see Section 2.2.2). Leaves from each sample were blotted dry, wrapped in paper towel and stored in paper envelopes, in case they were required at a later date.

Fungi consistently isolated from UK spinach samples were used in pathogenicity tests (Section 2.3).

2.2.2 Results and discussion

During the course of the project, 12 spinach samples were received from the UK (in addition to one from Portugal and 35 from Spain). Three red chard samples were received from the UK and two from Spain. The causal pathogens of leaf symptoms could not be determined in all cases because of disintegration during transit and growth of secondary pathogens and saprophytic fungi. Appendix 1 shows the range of pathogens isolated.

For UK spinach samples, fungi that were both found in association with leaf lesions and consistently isolated were *Stemphylium botryosum*, *Colletotrichum dematium* and two species of *Cladosporium* species. One *Cladosporium* isolate fitted the description of *C. variabile,* a species that is known to be pathogenic on spinach. The second species more closely fitted the description of *C. cladosporioides* which is recorded as a common secondary invader on a wide range of crop and weed hosts (Ellis, 1971). A *Fusarium* species was also isolated which most closely fitted the description of *F. avenaceum*, a cosmopolitan species (Nelson *et al.*, 1983) that is not recorded as a pathogen of spinach.

S. botryosum is known to be a causal agent of leaf spot in the USA (see Section 2.4.1) but has not previously been recorded as a pathogen of spinach in the UK. Following confirmation of pathogen identity by Central Sciences Laboratory,

the Plant Health and Seeds Inspectorate (PHSI) was informed. *Colletotrichum dematium* and *Cladosporium variabile* have previously been reported as pathogens of spinach in the UK.

S. botryosum (or the perfect stage *Pleospora herbarum*) was most frequently isolated from symptoms on samples from Spain. *Alternaria* sp. was also routinely isolated but was most likely due to secondary infection.

On red chard, the most common symptom type observed was reddish/purple pin-prick lesions. No fungal pathogens were consistently isolated from these symptoms and as the number of samples received was limited, no conclusions could be made about the relative importance of different leaf spot pathogens on UK chard crops.

Leaf spot symptoms consistently found on UK spinach samples are described below:

*2.2.2.1 Stemphylium leaf spot (*Stemphylium botryosum*)*
Lesions were pale brown (round or oval) with a green watersoaked margin (5-7.5 mm diameter) (Fig. 1). The outer edge of older lesions was better defined and there was often general chlorosis of surrounding leaf tissue and watersoaked leaf decay. Dark brown conidia were sometimes observed microscopically in the centre of leaf lesions (Fig. 2). Conidial dimensions were 15–23 (19.8) µm x 25–33 (27.8) µm. Ascostromata (of the fungal perfect stage) were occasionally observed in older lesions.

Conidial production occurred in new but not older cultures on PDA+S and V8 agar. Ascostromata developed in older cultures. The morphology of the fungus on spinach leaves and in culture corresponded with the published description for *Stemphylium botryosum* (teleomorph *Pleospora herbarum*) (Booth & Pirozynski, 1967). Pathogen identity was also confirmed at the Central Science Laboratory (C. Lane, pers. comm.).

Figure 1. Stemphylium leaf spot on spinach

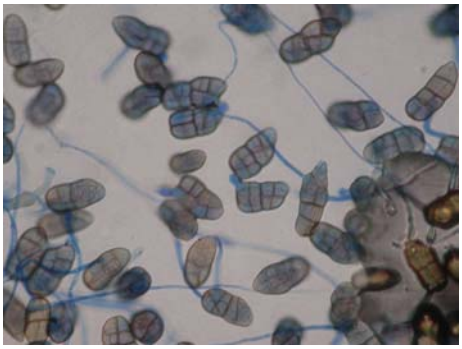

Figure 2. Conidia of *Stemphylium botryosum*

2.2.2.2 Anthracnose (Colletotrichum dematium*)*

Anthracnose caused by *Colletotrichum dematium* was confirmed on three spinach samples originating from the UK. Lesions were initially green, watersoaked and irregular in appearance, later turning light brown (up to 5 mm diameter) and merging with other lesions, some with chlorotic haloes and some with shot-holing (centre of lesions fallen out) (Fig. 3). Structures characteristic of *C. dematium* were visible within lesions under low power magnification; setae (black spiny protrusions) and acervuli (grey spore masses) were abundant.

In culture (on PDA+S), mycelium was dark olive green, with no aerial mycelium. The mycelium was granular in appearance due to abundant stromatic tissue. In order to induce sporulation in culture, isolates were sub-cultured onto ¼ strength PDA+S and incubated at approximately 18°C under UV light. Conidia were formed in grey acervuli (later turning salmon pink) and black setae were abundant. The conidia were hyaline, aseptate, uninuclear, falcate/fusiform and tapered at the ends. Dimensions were 22.5-27.5 (25.0) µm x 2.5 µm. The morphology corresponded with a published description for *Colletotrichum dematium* (Sutton, 1992).

Figure 3. Spinach anthracnose (*Colletotrichum dematium*)

2.2.2.3 Cladosporium leaf spot (Cladosporium variabile)

Leaf lesions were white, circular and up to 0.5 cm diameter (Fig. 4). Dark green sporulation was visible within the centre of lesions under low power magnification.

Cultures on PDA+S were dark olive green and mainly smooth but with sparse white spiralling aerial hyphae typical of *C. variabile* (Ellis, 1971) at the colony centres. Conidia dimensions were very variable, 17.5–42.5 (26.7) µm x 5.0–10.0 (7.8) µm. Conidia were 0–3 septate, pale brown, densely verrucose, and variable in shape (oblong with rounded ends, ellipsoidal or sub-globose).

Figure 4. *Cladosporium* leaf spot on spinach

2.3 Koch's postulates

2.3.1 Introduction

The pathogenicity of three fungal species isolated from spinach leaf spot symptoms was tested on detached spinach leaves.

2.3.2 Methods

Seeds of spinach (*Spinacea oleracea*) cv. Hector F1 (Johnsons Seeds) were sown in F1 compost in seed trays (approximately 40 seeds per tray). The trays were maintained in a polytunnel on a bench at ambient light and temperature and watered from above as required to maintain moist compost. Once the plants had reached the 2-3 true leaf stage, the trays were placed on Mypex matting outside.

The pathogenicity of the following fungi isolated from spinach leaf spot symptoms was tested:

Sample/isolate	Species	Type of material spores taken from
Control	Sterile distilled water	-
AR05/189	*Colletotrichum dematium*	Sporulating culture on ¼ PDA+S
AR06/172	*Stemphylium botryosum*	Leaf lesions and culture on PDA+S
AR06/173	*Stemphylium botryosum*	Leaf lesions and culture on PDA+S
AR06/174a	*Cladosporium variabile*	Culture on PDA+S

For each isolate, healthy detached leaves were placed individually in each of three sterile 9 cm diameter Petri dishes and five leaves were placed in a clear plastic box with lid (first wiped out with 90% ethanol), to give a total of eight leaves per sample.

A spore suspension of each isolate was made and filtered through a layer of muslin. For *C. dematium,* a spore suspension of 1 x 10^6 spores/ml was used; for the other isolates the spore suspension concentration was 1 x 10^4 spores/ml (due to limited sporulation on the cultures used).

For each isolate, ten 10 µl droplets of the spore suspension were pipetted onto each leaf. The Petri dishes were sealed with Parafilm and placed in the incubator (18-20°C, dark). The boxes were sealed with masking tape and placed on a laboratory bench not in direct sunlight (19-21°C).

The leaves were examined after 6 days. Fungal structures on developing lesions were examined microscopically and leaf tissue sections from the advancing edges of lesions were plated aseptically on to PDA+S, to check for the causal organism.

2.3.3 Results and discussion

Apart from slight yellowing and senescence of the leaves (also seen for other treatments), there was no distinct lesion development on the uninoculated control leaves.

AR05/189 (*Colletotrichum dematium*)

Lesions consistently developed at all of the inoculation points on all the leaves in Petri dishes and the plastic box (Fig. 5). The lesions were dark green with a brown water-soaked halo, approximately 0.75 cm in diameter. Setae were visible within lesions under low power magnification and conidia (typical of *C. dematium*) were abundant. *C. dematium* was isolated on PDA+S.

Figure 5. Lesion development on a spinach leaf after inoculation with *Colletotrichum dematium* (isolate code AR05/189)

AR06/72 (*Stemphylium botryosum*)

Distinct lesion development occurred in association with the majority of inoculation points (29 out of 30 points on leaves in Petri dishes and 49 out of 50 points on leaves in boxes). The lesions were grey/green, watersoaked and usually about 0.5 cm diameter (Fig. 6), although one lesion was 1 cm in diameter with mycelial growth visible. Dark brown Stemphylium spores were visible within lesions (confirmed microscopically) and there was sparse mycelial growth. *S. botryosum* was isolated on PDA+S.

Figure 6. Lesion development on a spinach leaf after inoculation with *Stemphylium botryosum* (isolate code AR06/72)

AR06/73 (*Stemphylium botryosum*)

Leaves in Petri dishes had brown speckled lesions in association with all of the inoculation points (similar to a hypersensitive response), and distinct lesion development was visible on 12 out of 30 inoculation points (Fig. 7). Leaves in boxes had distinct lesions on seven out of 50 inoculation points. Stemphylium spores were visible within lesions (confirmed microscopically) and there was sparse mycelial growth. *S. botryosum* was isolated on PDA+S.

Figure 7. Lesion development on a spinach leaf after inoculation with *Stemphylium botryosum* (isolate code AR06/73)

AR06/74 (*Cladosporium variabile*)

Leaves in Petri dishes had developed brown translucent lesions on nine out of 30 inoculation points (Fig. 8). Leaves in boxes had faint brown speckled lesions on 22 out of 50 inoculation points. A few *Cladosporium* spores were visible within lesions (confirmed microscopically). *C. variabile* was isolated on PDA+S.

Figure 8. Lesion development on a spinach leaf after inoculation with *Cladosporium variabile* (isolate code AR06/74a)

From these detached leaf studies, it was concluded that *Colletotrichum dematium* (AR05/189) and *Stemphylium botryosum* (AR06/72) were highly pathogenic on spinach. The second isolate of *S. botryosum* (AR06/73) was moderately pathogenic, while the isolate of *C. variabile* was only slightly pathogenic. Inoculation studies will be repeated more extensively during phase II of the project to include a wider range of isolates for each species, and using whole plant inoculations, in addition to detached leaf studies.

2.4 Biology and management of leaf spot diseases of spinach

A complex of fungi is associated with leaf spot symptoms on spinach (*Spinacea oleracea* L.), with *Stemphylium botryosum*, *Cladosporium variabile* and *Colletotrichum dematium* being the most commonly reported worldwide. The majority of studies on the biology and management of these leaf spot diseases have been done in the USA on spinach seed crops.

2.4.1 Stemphylium leaf spot

(*Stemphylium botryosum*; teleomorph *Pleospora herbarum*)

Stemphylium botryosum was first reported as a disease of spinach in California USA in 1997 (Koike *et al.*, 2001). Since that time, Stemphylium leaf spot has also been found in the states of Washington, Delaware, Maryland and Arizona (du Toit & Derie, 2001; Everts & Armentrout, 2001; Koike *et al.*, 2005). In the USA, Stemphylium leaf spot affects seed crops, resulting in reduced seed quality and yield. In addition, the disease reduces the quality of processing crops and may require additional hand sorting for fresh crops. Prior to this project, *Stemphylium* was suspected as a cause of leaf spot in the UK but not officially recorded (C. Lane, CSL, pers. comm.).

2.4.1.1 Symptoms

Initial symptoms are small (2-5 mm diameter) circular to oval, grey-green leaf spots, which develop about one week after infection has taken place. As the disease develops, the lesions enlarge, coalesce and turn light brown in colour and papery in texture (Koike & Henderson, 2001). Papers from the USA suggest that fungal structures are not usually visible within Stemphylium leaf spots and this helps to distinguish the disease from other foliar diseases such as *Cladosporium* leaf spot (dark green spore production), downy mildew (purple fungal growth) and anthracnose (dark sporing structures). However, during this project, clusters of dark brown Stemphylium spores could occasionally be seen on the lesion surface. Symptoms observed on baby leaf spinach due to *S. botryosum* typically had a green watersoaked margin to the lesions.

More developed symptoms can closely resemble the tan, circular spots caused by pesticide or fertiliser damage, and for this reason the disease may commonly be incorrectly diagnosed.

2.4.1.2 Sources of the disease

<u>Seed</u>

Research in the USA has demonstrated that *S. botryosum* is seed-borne surviving either as mycelium and conidia, or as survival structures (pseudothecia) of the teleomorph (*Pleospora herbarum*). *S. botryosum* was present in each of 77

spinach seed lots tested (produced in the US, Denmark, the Netherlands, or New Zealand in 2000 to 2003) at a mean incidence of 29% (Hernandez-Perez & du Toit, 2006). The same authors found that the percentage of seed infected with *S. botryosum* declined with time but that the pathogen remained viable on spinach seed (stored at optimum temperatures for maintaining seed viability; 4.4°C and 60% relative humidity) for up to 11 years. Isolates of *S. botryosum* from seed were pathogenic on spinach (Hernandez-Perez & du Toit, 2006) and transmission of *S. botryosum* from infected seeds to seedlings was demonstrated in glasshouse experiments (Hernandez-Perez & du Toit, 2005). As well as being detected in the seed coat or pericarp, internal infection by *S. botryosum* in seed embryos was also detected (Hernandez-Perez & du Toit, 2006). This internal seed infection by the pathogen suggests there could be potential difficulties in eradicating *S. botryosum* from infected spinach seed using seed treatment. The prevalence of *S. botryosum* in spinach seed lots, combined with routine international movement of spinach seed, may explain widespread first outbreaks of Stemphylium leaf spot in several US states, plus observations of the disease during this project on samples from Spain and the UK.

Alternative hosts

Stemphylium botryosum has been reported as a pathogen on a wide range of hosts (including lettuce, pea and onion) and is also a common saprophyte, for example surviving on dead or damaged sugar beet material. In pathogenicity studies, Koike *et al.* (2001) found that four isolates of *S. botryosum* from spinach caused typical leaf spot symptoms on spinach but did not infect 21 other hosts tested (including closely related hosts such as sugar beet, beet and a chenopod weed species). It was concluded that this pathogen 'type' has a narrow host range and does not infect plants other than spinach.

Crop debris

S. botryosum can survive on spinach crop debris. For example, *P. herbarum*, the teleomorph of *S. botryosum* was isolated consistently from spinach seed stalk debris and found to be pathogenic on spinach (du Toit & Derie, 2003). Fruiting bodies (pseudothecia) on crop debris discharge airborne ascospores which could be a primary source of inoculum in spring for infection of newly-planted spinach crops (du Toit & Derie, 2004). The same authors found that the viability of fruiting bodies on crop debris could be reduced to 10 weeks or less by debris burial in soil, compared to survival for at least 15 weeks on debris at the soil surface.

2.4.1.3 Conditions for infection and spread

Infection may take place over a wide range of conditions but is favoured by temperatures in the range 18-24°C and prolonged periods of leaf wetness. Spores may be spread by wind, rain splash, irrigation, and farm implements or workers.

Spinach seed growers in Washington State USA observed that leaf spot develops most rapidly after antithesis. Based on this observation, experiments demonstrated

that the presence of pollen on spinach leaves enhances disease development by *S. botryosum* (du Toit & Derie, 2002).

2.4.1.4 Disease management

Because of the seed-borne nature of Stemphylium leaf spot, seed needs to be clean from *S. botryosum*. Seed can be tested for the presence of *S. botryosum* using a published freeze-blotter seed health assay (du Toit *et al.*, 2005). Although some seed companies use hot water and/or chlorine treatment for spinach seed, the details of these seed treatment protocols are proprietary. A recent paper (du Toit & Hernandez-Perez, 2005) reports on the efficacy of chlorine and hot water for eradication of fungal pathogens on spinach seed. Surface sterilisation in 1.2% NaOCl for more than 10 minutes reduced the incidence of *S. botryosum* to <20% but did not eliminate the fungus, even after 60 min. Seed germination was not adversely affected. Hot water treatment of spinach seed significantly reduced the incidence of *S. botryosum* but conditions required to eliminate the pathogen even from a lightly infected seed lot (5%), adversely affected seed germination. The difficulty in eradicating *S. botryosum* from seed by disinfection or hot water treatment may be due to the deep-seated nature of the infection.

Little is known about the variation in resistance of commercial spinach cultivars to *S. botryosum*. Research in California showed only slightly less lesion development on the savoy spinach cultivar 'Vienna' than on other varieties tested including flat leaf and semi-savoy types (Koike *et al.*, 2001).

Cultural practices to reduce the risk of Stemphylium leaf spot development include, incorporation of spinach residues/debris after harvest to accelerate microbial breakdown, and management of irrigation (where used) to reduce leaf wetness duration.

On a spinach seed crop in Washington State, fungicides with the following active ingredients gave the most effective control of Stemphylium and *Cladosporium* leaf spots: azoxystrobin, pyraclostrobin, pyraclostrobin+boscalid, iprodione, and kresoxim-methyl, reducing severity of leaf spots from 19% to 2% or less (du Toit *et al.*, 2003). Chlorothalonil and mancozeb were not found to be effective against *S. botryosum* (du Toit & Derie, 2002).

2.4.2 Cladosporium leaf spot

(Cladosporium variabile)

This disease is frequently observed on spinach and has been reported from USA, Europe (including the UK) and Asia. In the UK, it is reported to be more severe in cool, wet autumn conditions. *Cladosporium* leaf spot is caused by *C. variabile.* Another *Cladosporium* species, *C. macrocarpum*, which is a common saprophyte, may also be found on deteriorating spinach leaves and on spinach seed but is not a pathogen of spinach (Ellis, 1971).

2.4.2.1 Symptoms

Typical lesions are usually round, white/yellow with a dark margin and up to 1 cm in diameter, becoming irregular in shape when they merge with other lesions. Dark green spores and mycelium later develop in the centres of these lesions. The dark green sporulation distinguishes *Cladosporium* leaf spot from symptoms of anthracnose and Stemphylium leaf spot. The disease can be particularly severe on seed crops, in which ripening seed may also develop lesions and become shrivelled.

2.4.2.2 Sources of the disease

Hernandez-Perez & du Toit (2006) detected *C. variabile* in 48% of 77 spinach seed lots produced in Denmark, the Netherlands, New Zealand, or the USA in 2000 to 2003. Hernandez-Perez & du Toit (2005) demonstrated seed transmission of the fungus in greenhouse trials but this has not been proven under field conditions.

Volunteer spinach can serve as a 'reservoir' for the disease. Typical symptoms of *Cladosporium* leaf spot were observed on volunteer spinach following a severe outbreak of the disease. *C. variabile* isolated from the symptoms was pathogenic on spinach (du Toit & Derie, 2003).

C. variabile is reported to be pathogenic to sugar beet (*Beta vulgaris*) and *Chenopodium amaranticolor* as well as spinach, but it is not clear whether cross infection would occur under field conditions (Fuentes-Dávila & Gabrielson, 1994).

2.4.2.3 Conditions for infection and spread

The fungus can grow and infect spinach under a wide range of temperatures, but a temperature range of 15-20°C with relative humidity above 80% is most conducive to disease development (Fuentes-Dávila & Gabrielson, 1994). Spores of *C. variabile* can germinate and penetrate leaf stomata within 48 h of inoculation in the presence of free moisture and symptoms of the disease generally follow 4-10 days later (Inglis *et al.*, 1997; du Toit & Derie, 2002). Once infection is established, the fungus grows in the leaf tissue and spores produced within leaf lesions start new infection cycles. Spores of *C. variabile* can be spread by wind, rain splash or carried on equipment.

2.4.2.4 Disease management

Because of the seed-borne nature of *Cladosporium* leaf spot, seed needs to be clean from *C. variabile*. Seed can be tested for the presence of *C. variabile* using a published freeze-blotter seed health assay (du Toit *et al.*, 2005). Although some seed companies use hot water and/or chlorine treatment for spinach seed, the details of these seed treatment protocols are proprietary. A recent paper (du Toit & Hernandez-Perez, 2005) reports on the efficacy of chlorine and hot water for eradication of fungal pathogens on spinach seed. Using precisely controlled conditions, chlorine (1.2% NaOCl for □20 min) or hot water seed treatments

(50°C water for 20 min) can be used to eradicate *C. variabile* without damaging seed germination.

As volunteer spinach can act as a source of inoculum for *Cladosporium* leaf spot, this should be destroyed.

In the USA, some semisavoy types like 'Ozarka II' and 'Fall Green' have been shown highly susceptible to *Cladosporium* leaf spot in greenhouse tests and field observations. The savoy cultivar Winter Bloomsdale tended to be more resistant. No commercially acceptable level of resistance has been identified (Inglis *et al.*, 1997)

On a spinach seed crop in Washington State, fungicides with the following active ingredients gave the most effective control of Stemphylium and *Cladosporium* leaf spots: azoxystrobin, pyraclostrobin, pyraclostrobin+boscalid, iprodione, and kresoxim-methyl, reducing severity of leaf spots from 19% to 2% or less (du Toit *et al.*, 2003). Chlorothalonil and mancozeb were not found to be effective against *S. botryosum* (du Toit & Derie, 2002).

2.4.3 Anthracnose

(*Colletotrichum dematium*, *C. dematium* f. sp. *spinaciae*)

Spinach anthracnose has been previously reported and is occasionally observed in the UK. There were at least two confirmed outbreaks in October 2005. The disease has been reported from most spinach production areas in the US and also from mainland Europe but is considered only a sporadic problem (Correll *et al.*, 1994). There was also a recent first report of anthracnose on baby-leaf spinach in Australia (Washington *et al.*, 2006)

2.4.3.1 Symptoms

Initial symptoms are small, circular, water-soaked green lesions on both young and old leaves (Correll *et al.*, 1994). Lesions enlarge and become chlorotic or necrotic. Older lesions turn light brown and affected leaf tissue becomes thin and papery. Lesions may coalesce so that the entire leaf is affected. A useful diagnostic feature of the disease is that dark spore-containing bodies (acervuli) are often observed in anthracnose lesions. Under the microscope, abundant dark protrusions (setae) can be seen in association with the acervuli. These features can be used to distinguish anthracnose from *Cladosporium* or Stemphylium leaf spot diseases, both of which also form circular lesions.

2.4.3.2 Sources of the disease

The fungus survives as dormant mycelium in infected plant debris and this is probably the primary source of inoculum for spinach anthracnose. Infected volunteer plants could also be a source of the disease. *C. dematium* can be seed-borne on spinach, with Hernandez-Perez & du Toit (2006) reporting low level seed

contamination (0.04%) in two of 27 seed lots from Denmark and one of six seed lots from the Netherlands in 2003. However, the relative importance of seed-borne inoculum in outbreaks of anthracnose on spinach has not been documented.

C. dematium isolated from spinach is generally thought to be pathogenic only to spinach, hence classification of isolates *ex* spinach as *C. dematium* f. sp. *spinaciae* by some authors. However, Washington *et al.* (2006) reported that while six spinach cultivars were highly susceptible to an isolate of *C. dematium* from spinach, four beet cultivars were also slightly susceptible.

2.4.3.3 Conditions for infection and spread

Conditions favouring infection by *Colletotrichum* spp. include high relative humidity and leaf wetness. Once infection has taken place, spores develop in structures (acervuli) on the leaf surface and are readily spread to neighbouring plants or beds by overhead irrigation, rain-splash or wind-driven rain, resulting in rapid disease spread. Anthracnose epidemics are sporadic but factors such as dense plantings, poor air circulation and low plant fertility can increase the risk of infection (Correll *et al.*, 1994)

2.4.3.4 Disease management

Host resistance to anthracnose has been reported (Correll *et al.*, 1993) but is not available as a trait in commercial cultivars.

Cultural practices to reduce the risk of anthracnose development include:

- Removal or deep burial of crop debris after harvest
- Rotation of at least three years
- Management of irrigation (where used) to reduce leaf wetness duration
- Eliminate volunteer spinach
- Adequate plant nutrition.

The activity of approved fungicides for control of spinach anthracnose is not well-documented. Once symptom development has occurred, the disease can spread rapidly under wet conditions. On farms where anthracnose has been confirmed as a recurring problem, use of a protectant fungicide such as copper may be warranted. Copper fungicides may delay epidemic development, but are unlikely to give adequate control under continuous wet conditions.

2.4.4 Other leaf spot pathogens

Cercospora beticola, *Ramularia beticola* and *Phoma betae* can cause leaf spots on spinach (Foister, 1961; Correll *et al.*, 1994). During this project, one spinach sample was received from Spain with *Cercospora* leaf spot. Symptoms were orange-brown lesions, often collapsed in the centre ('shot-holed') less than 0.5

cm diameter, with a yellow halo. Black dots (stromata) were visible within the lesions and are a useful diagnostic feature for cercospora leaf spot. *C. beticola*, *R. beticola* and *P. betae* are more commonly found on *Beta vulgaris* and sub-species, so the biology and management of these diseases are reviewed under diseases of chard in Section 2.5. *Alternaria* species have also occasionally been reported as causing leaf spots of spinach in other countries. A bacterial leaf spot of spinach (*Pseudomonas syringae* pv. *spinaciae*) has been described as a minor problem from Europe (Italy) and the USA (Bazzi *et al.*, 1988; Koike *et al.*, 2002).

2.4.5 Leaf spot pathogen interactions

There are general observations in the literature that spinach leaf spots are due to a 'complex' of pathogens. Specific reports on pathogen interactions are follows:

- *C. variabile* and *S. botryosum* are each pathogenic on spinach but *S. botryosum* is reported to cause more severe foliar damage than *C. variabile*. There does not seem to be a synergistic interaction between the two fungi (du Toit & Derie, 2002).
- In this project, *Cladosporium* sp. and *Stemphylium botryosum* were isolated from lesions on the same spinach leaf.
- *Colletotrichum dematium* is a primary pathogen causing anthracnose on spinach, but it is also a common secondary pathogen, readily colonising lesions caused by other diseases such as white rust (Correll *et al.*, 1994).
- Foliar damage from infection due to downy mildew may create sites for leaf spot infection.
- It was observed in this project that in baby- and mid-leaf spinach crops, symptom development does not always follow typical descriptions of the same diseases on spinach seed crops described in the literature. Often, because of the soft and succulent nature of the young leaf tissue, development of individual lesions is rapidly followed by general disintegration and rotting of leaf tissue.

2.5 Knowledge gaps

Gaps in knowledge on the biology and management of spinach leaf spot in the UK have been identified as follows:

- The seed-borne nature of *S. botryosum*, *C. dematium* and *C. variabile* has been reported in scientific literature. However, the presence of these pathogens in seed lots of varieties widely used for baby leaf spinach production in the UK has 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 and *Cladosporium* leaf spot symptoms were observed on spinach volunteers. However, the role of infected crop debris in the development of leaf spot diseases in intensive baby-leaf spinach production under UK conditions needs to be ascertained.
- It is not known whether the risk of leaf spot diseases developing is increased by re-use of crop meshes on subsequent crops. This topic is the subject of a separate HDC project (FV 283).
- Lesions due to 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, such as Lazio, Whale, Falcon, Tarpy and Compania.
- There is currently little scope for chemical control of spinach leaf spot diseases because of limited product approvals and MRLs. Fungicides approved for control of downy mildew in the UK (fosetyl-aluminium and metalaxyl-M) are generally ineffective against leaf spot pathogens. A protectant fungicide such as Amistar (azoxystrobin) could minimise onset of infection, but has limited eradicant activity and there are reports of phytotoxicity. There may be potential for disease control using fungicides such as Signum (boscalid + pyraclostrobin) which gave promising results on seed crops in the USA, or new products such as Switch (cyprodinil + fludioxonil) (not currently approved for use on spinach). The efficacy of a range of fungicides for management of spinach leaf spots will be evaluated in Phase II of the project.
- 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.

2.6 Biology and management of leaf spot diseases of chard

Chard is in the beet family (*Beta vulgaris* subsp. *vulgaris*) and is prone to the same leaf spot diseases that affect sugar beet and other beet crops in the UK. The information for this section was sourced from Whitney & Duffus (1986), Sherf & Macnab (1986) and US University extension websites such as www.ag.ndsu.nodak.edu.

2.6.1 Cercospora leaf spot
(Cercospora beticola)

2.6.1.1 Symptoms
Symptoms develop as circular leaf spots with light to tan centres and a red-purple margin. Minute black dots (stromata) form during humid weather in leaf spots, on crop debris or newly infected leaves; they can be seen within the lesions using a hand lens. In warm weather, the stromata produce greyish spores, which give the leaf spot a fuzzy appearance. Leaf spots can merge and kill large areas of leaf tissue.

2.6.1.2 Sources of the disease
The pathogen can be seed-borne but usually on the seed surface. It can over-winter on crop debris as well as surviving on crop and weed hosts (e.g. red beet, sugar beet, chard, perpetual spinach, chenopod weeds). The pathogen can survive 12 to 18 months even if buried 30-45 cm in soil

2.6.1.3 Conditions for infection and spread
Cercospora spores form most readily at 20-26°C at relative humidities of 90-100% (formation inhibited at temperatures of less than 10°C), and are spread by splashing water, wind and insects. Spores germinate and infect leaves (via stomata) at daytime temperatures above 25°C and night temperatures above 15°C, and high relative humidities (90-95%) or free moisture. Infection is reduced or inhibited at temperatures less than 15°C or when leaf wetness duration is less than 11 hours. Leaf spots develop from 5 to 21 days after infection, depending on inoculum amount, temperature and leaf wetness duration.

2.6.1.4 Disease management
Cultural practices include the following:

- Bury or burn off crop residues
- Avoid planting chard in close proximity to sugar beet crops
- Seed testing
- There are currently no approved fungicide seed treatments for chard.

2.6.2 Ramularia leaf spot

(*Ramularia beticola*)

2.6.2.1 Symptoms

Symptoms appear as light brown leaf spots that are angular, and larger on older leaves, sometimes with a dark border. Silvery grey to white spore clusters are typically visible within lesions and these can be used to distinguish the disease from *Cercospora* leaf spot. Severe infections can cause the leaves to dry out entirely.

2.6.2.2 Sources of the disease

The fungus is possibly seed-borne. It can persist in soil and crop debris for over two years, and can also survive on alternative crop and weed hosts (e.g. red beet, sugar beet, chard, perpetual spinach, chenopod weeds).

2.6.2.3 Conditions for infection and spread

Clusters of spores can develop as soon as RH is over 70% and the temperature between 5-25°C, with the optimum at 16-17°C. Conidial germination and penetration of the leaf epidermis takes 2-3 days if RH is over 95% and the symptoms show within the following 15 days. Spores are air-borne.

2.6.2.4 Disease management

As for cercospora leaf spot

2.6.3 Phoma leaf spot

(*Phoma betae*)

2.6.3.1 Symptoms

Symptoms appear as round leaf spots with a dark margin, sometimes with concentric rings. The fungus can also cause seedling black leg and crown rot. Black fruiting bodies (pycnidia) may be visible within lesions.

2.6.3.2 Sources of the disease

The fungus remains alive in seed as long as the seed remains viable, but seed infection is much reduced after 4 years. The fungus grows from the cotyledon into the seedling and causes damping-off. It can persist in soil and crop debris (for 26 months after harvest), and can also survive on alternative crop and weed hosts (e.g. red beet, sugar beet, chard, perpetual spinach, chenopod weeds).

2.6.3.3 Conditions for infection and spread

Phoma grows between 2 and 35°C with the optimum at 24°C. Host penetration and colonisation is best at lower temperatures, between 7 and 13°C. The optimum temperature for pycnidial production is 27 to 29°C. Spores are spread by rainsplash.

2.6.3.4 Disease management

As for cercospora leaf spot.

2.7 Host range of leaf spot pathogens

Table 1. Pathogenicity of fungal leaf spot pathogens on crop hosts of the *Chenopodiacae* family (based on literature reviewed for Sections 2.4 and 2.5)

Pathogen	Crop host			
	Spinach (*Spinacea oleracea*)	Red chard (*Beta vulgaris*)	Spinach beet (*Beta vulgaris*)	Sugar beet (*Beta vulgaris*)
Stemphylium botryosum	✓	X	X	X*
Cladosporium variabile	✓	?	?	?
Colletotrichumdematium	✓	?	?**	?
Cercospora beticola	✓	✓	✓	✓
Ramularia beticola	✓	✓	✓	✓
Phoma betae	✓	✓	✓	✓

*can be a common saprophyte on dead or damaged plant material

** reported in Defra DOVE project in an organic spinach beet crop

2.8 Overall conclusions

- Leaf spots on baby leaf spinach in the UK were found to be due to *Stemphylium botryosum*, *Colletotrichum dematium* and *Cladosporium variabile*.
- These three pathogens are known to be seed-borne.
- *Stemphylium botryosum* has not previously been reported as a pathogen of spinach in the UK.
- Spinach leaf spots due to different fungi were visibly similar and were difficult to diagnose by visual examination only. Because of the fragile nature of baby leaf spinach, leaves with lesions were prone to secondary infection and general leaf deterioration quickly occurred. Some Stemphylium leaf spots could be confused with pesticide scorch symptoms.
- From inoculation studies using detached spinach leaves, it was concluded that *Colletotrichum dematium* (isolate AR05/189) and *Stemphylium botryosum* (isolate AR06/72) were highly pathogenic on spinach. A second isolate of *S. botryosum* (isolate AR06/73) was moderately pathogenic, while an isolate of *C. variabile* was only slightly pathogenic. Inoculation studies will be repeated more extensively during phase II of the project to include a wider range of isolates for each species, and using whole plant inoculations, in addition to detached leaf studies.
- Insufficient samples of red chard were received to determine the major leaf spot pathogens affecting this crop. Cercospora, *Ramularia* and Phoma leaf spots of chard are most commonly reported in literature.

A knowledge review on spinach leaf spots completed in phase I of the project confirmed the following:

- *S. botryosum*, *C. variabile* and *C. dematium* can all survive on seed. Transmission from seed to emerging plants has been demonstrated experimentally for *S. botryosum* and *C. variabile* but not for *C. dematium*.
- Seed treatment methods (hot water treatment and disinfectants) for controlling seed-borne diseases of spinach have been investigated.
- Other sources of inoculum for the diseases on spinach in the USA include woody crop debris (for *S. botryosum* and *C. dematium*) and volunteer plants (*C. variabile*).
- Isolates of *S. botryosum* from spinach were only pathogenic to spinach and did not infect other hosts (including closely related crop and weed hosts). *C. variabile* and *C. dematium* from spinach were slightly pathogenic on beet in laboratory tests but cross-infection in the field has not been demonstrated.
- Environmental conditions: Infection by *S. botryosum* may take place over a wide range of conditions but is favoured by temperatures in the range 18-24°C and prolonged leaf wetness periods. For *C. variabile*, a temperature range of 15-20°C with relative humidity above 80% is most conducive to disease

development. Conditions favouring infection by *Colletotrichum* species include high relative humidity and leaf wetness.

- Little is known about the variation in resistance of commercial spinach cultivars to *S. botryosum*, *C. dematium* or *C. variabile.*
- On spinach seed crops in Washington State (USA), fungicides with the following active ingredients gave the most effective control of Stemphylium and *Cladosporium* leaf spots: azoxystrobin, pyraclostrobin, pyraclostrobin + boscalid, iprodione, and kresoxim-methyl, reducing the severity of leaf spots from 19% to 2% or less (du Toit *et al.*, 2003). Fungicide activity against spinach anthracnose has not been reported.

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2.10 Technology transfer

- Telephone and email responses to growers requesting information on sample diagnosis in 2004, 2005 and 2006.
- Green KR. 2006. Leaf spots on spinach. *HDC Factsheet (in prep.)*
- Green KR. 2006. *HDC News* article *(in prep.)*

2.11 Acknowledgements

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3 APPENDIX 1

Description of spinach and chard samples received (2004-2006)

Sample Reference	Date received	Crop	Region/ Country	Brief symptom description	Fungi isolated
AR04/215	22-Nov-04	Spinach	Spain	Circular spots, watersoaked area around spot	*Stemphylium botryosum*
AR04/221	1-Dec-04	Spinach	Portugal	Pale, tan lesions, no water soaking	-
AR05/003	18-Jan-05	Spinach	Spain	Pale, tan lesions, no water soaking	*Alternaria* sp.*
AR05/005	25-Jan-05	Spinach	Spain	Pale - dark brown lesions	*Botrytis* sp.*
AR05/011	8-Feb-05	Spinach	Spain	Light yellow circular spots	*Alternaria* sp.*
AR05/014	18-Feb-05	Spinach	Spain	Pale, tan lesions, no water soaking	*Alternaria* sp.*
AR05/018	1-Mar-05	Spinach	Spain	Light brown lesions, green water soaked area on outer edge of spot	*Alternaria* sp.*
AR05/022	7-Mar-05	Spinach	Spain	Leaf chlorosis, parchment coloured spots	*Alternaria* sp.*
AR05/023	7-Mar-05	Spinach	Spain	Leaf chlorosis, parchment coloured spots	*Alternaria* sp.*
AR05/026	17-Mar-05	Spinach	Spain	Light brown lesions, water soaked area on outer edge of spot	*Fusarium* sp.* / *Stemphylium botryosum*
AR05/029	21-Mar-	Spinach	Spain	Leaf chlorosis, parchment coloured spots (Some with	*Stemphylium botryosum*

	05			dark haloes)	
AR05/030	21-Mar-05	Spinach	Spain	Inter-vein leaf chlorosis, few scattered small, dark brown spots	*Alternaria* sp.* / *Fusarium* sp.*
AR05/033	24-Mar-05	Spinach	Spain	Pale, tan lesions. Few dark brown, slightly elevated spots.	*Pleospora herbarum*
AR05/038	5-Apr-05	Spinach	Spain	Pale, tan lesions. Some surrounded by black pycnidia	*Pleospora herbarum*
AR05/039	5-Apr-05	Spinach	Spain	Grey - pale brown circular lesions (4-8mm in diam)	*Stemphylium botryosum*
AR05/040	5-Apr-05	Spinach	Spain	Grey – brown lesions. Dark brown spot at centre of lesion.	*Alternaria* sp.*
AR05/049	11-Apr-05	Spinach	Spain	Pale, tan lesions	*Pleospora herbarum* / *Fusarium* sp.*
AR05/051	11-Apr-05	Spinach	Spain	Pale, tan lesions	*Stemphylium botryosum* / *Alternaria* sp.*
AR05/052	11-Apr-05	Spinach	Spain	Pale, tan lesions	*Alternaria* sp.*
AR05/058	18-Apr-05	Spinach	UK	Irregular shaped dry patches on leaves	*Rhizopus* sp.*
AR05/059	15-Apr-05	Spinach	Spain	Irregular shaped brown lesions with chlorotic haloes	*Stemphylium botryosum*
AR05/060	18-Apr-05	Spinach	Spain	Slightly raised, pale-tan lesions	*Pleospora herbarum*
AR05/061	18-Apr-05	Spinach	Spain	Regular shaped white spots	-
AR05/062	18-Apr-	Spinach	Spain	Regular shaped white spots	-

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AR05/063	18-Apr-05	Spinach	Spain	Large patches of dead leaf tissue.	*Pleospora herbarum*
AR05/064	18-Apr-05	Spinach	Spain	Pale coloured, large oval lesions surrounded by darker perimeter	*Fusarium* sp. / *Alternaria* sp.*
AR05/072	25-Apr-05	Spinach	Spain	Large circular lesions. Pale colour. 1 cm diameter.	*Stemphylium botryosum* / *Fusarium* sp.*
AR05/073	25-Apr-05	Spinach	Spain	Regular shaped white spots	-
AR05/081	9-May-05	Spinach	Spain	Regular shaped white spots	*Fusarium* sp.*
Sample Reference	Date received	Crop	Region/ Country	Brief symptom description	Fungi isolated
AR05/085	11-May-05	Spinach	Spain	Random, pale coloured spots	-
AR05/088	16-May-05	Spinach	Spain	Large, irregular shaped lesions devoid of chlorophyll.	*Fusarium* sp.*
AR05/089	18-May-05	Spinach	Spain	-	-
AR05/093	23-May-05	Spinach	Spain	Pale, regular shaped lesions. 1-3mm in diameter. Approx. 10 per leaf.	-
AR05/148	11-Aug-05	Spinach	West Sussex, UK	Large yellow lesion (2-5cm +), circular necrotic tissue to centre	*Stemphylium botryosum*
AR05/164	13-Sep-05	Spinach	Thames Valley, UK	1.5cm diam. Pale brown circular spot. Well defined outer edge	*Stemphylium botryosum*
AR05/176	29-Sep-	Spinach	UK	Pale irregular lesions with defined edges	*Cladosporium* sp.

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AR05/178	30-Sep-05	Spinach	Dorset, UK	Pale lesions, regular and round. Watersoaked border.	*Stemphylium botryosum* & *Cladosporium* sp.
AR05/182	10-Oct-05	Spinach	UK	Yellow/grey blotches. Trace of grey/purplish sporulation on underside	*Fusarium* sp.*
AR05/189	20-Oct-05	Spinach	Hampshire, UK	Green watersoaked lesions and pale brown lesions	*Colletotrichum dematium*
AR05/192	28-Oct-05	Spinach	Hampshire, UK	Pale yellow/brown irregular lesions. Slight sporulation on underside	*Peronospora farinosa*
AR05/193	31-Oct-05	Spinach	Dorset, UK	Pale brown lesions with chlorotic haloes, some shot-holing	*Colletotrichum dematium*
AR05/200	29-Nov-05	Spinach	Spain	Orange/brown lesions with shotholing and black stromata	*Cercospora beticola*
AR06/002	23-Jan-06	Spinach	Spain	Small circular spots on leaves	*Cladosporium* sp & *Stemphylium botryosum*
AR06/003	23-Jan-06	Spinach	Spain	Many, small circular spots on leaves	*Cladosporium* sp & *Stemphylium* sp.
AR06/010	3-Feb-06	Spinach	Spain	Numerous, yellow circular patches with brown points in centre.	*Cladosporium* sp.
AR06/072	11-May-06	Spinach	Hampshire, UK	Tan lesions, with green watersoaked margin, dark brown sporulation in centre	*Stemphylium botryosum*
AR06/073	11-May-06	Spinach	Hampshire, UK	Tan lesions, with green watersoaked margin, dark brown sporulation in centre	*Stemphylium botryosum*
AR06/074	11-May-06	Spinach	Hampshire, UK	White circular lesions with dark green sporulation in lesion centre Tan lesion with yellow halo and shot-holed, setae	*Cladosporium variabile* & *Colletotrichum dematium*

				visible	
AR05/050	11-Apr-05	Red Chard	Spain	Small, red, circular spots around leaf margin	*Alternaria* sp.*
AR05/071	25-Apr-05	Red Chard	Spain	Circular spots surrounded by red outline	-
AR05/167	14-Sep-05	Red Chard	UK	Purple spotting	-
AR05/180	7-Oct-05	Red Chard	East Anglia, UK	Red pin-prick lesions, slightly raised.	-
AR05/190	21-Oct-05	Red Chard	East Anglia, UK	Pale brown lesions with dark red or black border	*Alternaria* sp.*

*Probable secondary infection

Pleospora herbarum is the perfect stage of *Stemphylium botryosum*