Project title:	Spinach: biology and integrated management of leaf spot – Phase II
Project number:	FV 268a
Project leader:	Dr Kim Green, ADAS
Report:	Final report
Previous reports	Final report for FV 268 Annual report for FV 268a
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Project coordinator:	Mr Shaun Clarkson
Date project commenced:	1 April 2006
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Key words:	Spinach, leaf spots, pathogens, *Stemphylium botryosum*, *Cladosporium variabile*, *Colletotrichum dematium*, anthracnose

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 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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GROWER SUMMARY

Headline

- Two applications of Signum (9 and 14 days after sowing) controlled leaf spot until the harvest date despite high disease pressure.
- Leaf wetness durations of 24 hours or more resulted in higher incidence and severity of leaf spot diseases.
- Seed was identified as an important source of infection for Stemphylium leaf spot (*Stemphylium botryosum*) *in Spinach*
- Spinach crop debris and volunteers are the most likely sources of infection for anthracnose while seed and infested crop debris may cause outbreaks of 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. Compare the susceptibility of widely used baby-leaf spinach varieties to three selected leaf spot pathogens.

5. Screen foliar fungicides and other novel products for their relative protectant and eradicant activity against three spinach leaf spot pathogens, in inoculated pot trials.
6. Evaluate promising products for the control of spinach leaf spot diseases in an inoculated field experiment.
7. 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

Studies in project FV 268 and subsequently in FV 268a demonstrated that leaf spots on baby leaf spinach in the UK can be caused by *Colletotrichum dematium* (anthracnose), *Cladosporium variabile* (Cladosporium leaf spot) and *Stemphylium botryosum* (Stemphylium leaf spot). During the project, attempts to produce sporulating cultures of *S. botryosum* have been only partially successful, despite testing a range of techniques used by other researchers. For this reason, results on varietal susceptibility and fungicidal control (experiments requiring artificial inoculation using sporulating cultures) have been limited to anthracnose and Cladosporium leaf spot only. Experiments on seed-borne inoculum and effects of environmental conditions on leaf spot development have been inclusive of all three pathogens.

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. Twenty-one spinach seed lots (17 varieties) were tested during the project, covering two seed harvest seasons, with 19 lots produced in Northern Europe and two lots produced in New Zealand. The seed was provided by three different companies. *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 19 out of 21 seed lots, with percentage incidence ranging from 0.8 to 59%. Stemphylium isolates from seed were pathogenic to spinach. *C. variabile* affected

2 out of 21 seed lots (incidences of 0.3 and 1.3%), and isolate pathogenicity was not demonstrated. *Colletotrichum dematium* (spinach anthracnose) was not detected in any of the seed lots. *Verticillium dahliae* was also detected in 15 seed lots tested (0.3 – 52% incidence). 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 infection 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 if crops are not thoroughly destroyed after harvest.

Crop debris

A pot experiment was set up to determine the longevity of survival of *C. dematium* (anthracnose) and *C. variabile* (Cladosporium leaf spot) on spinach debris either on the soil surface or buried, and stored either wet or dry. Both fungi survived under certain conditions on spinach crop debris for a period of at least 12 months. *C. dematium* survived both on the soil surface and to a depth of 15 cm, under both wet and dry soil conditions. *C. variabile* remained viable for up to 6 months on the soil surface and buried; after this period it was only recovered from debris that had remained continually dry on the soil surface, which is unlikely to occur under normal field conditions.

From experimental results it is concluded that the following sources of inoculum are most likely to be contributing to outbreaks of individual leaf spot diseases on UK baby-leaf spinach:

- *Stemphylium botryosum* (Stemphylium leaf spot) – seed (high proportion of seed lots infested)
- *Cladosporium variabile* (Cladosporium leaf spot) – seed (low proportion of seed lots infested) and infested debris (up to 6 months)
- *Colletotrichum dematium* (anthracnose) – infested debris (at least 12 months) and volunteer plants

Varietal susceptibility

In project year 1, 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. 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. 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.

Effect of environmental conditions

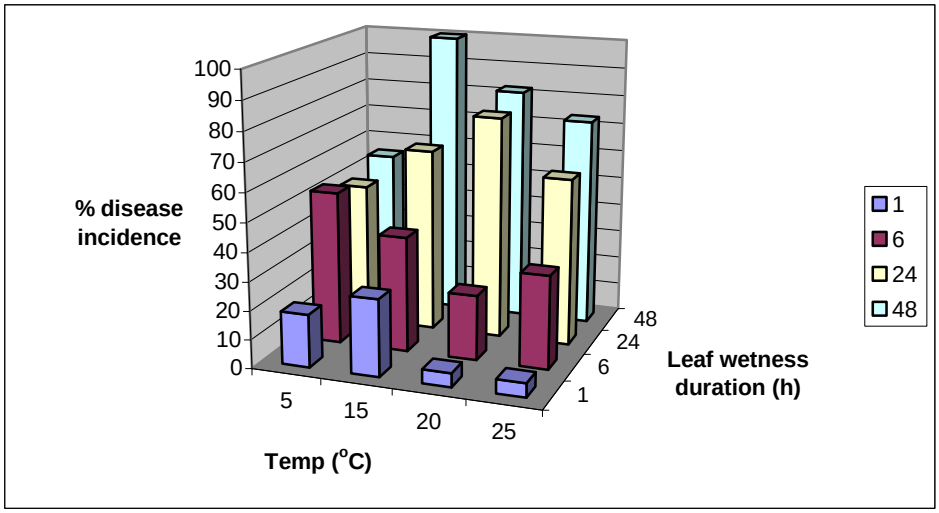
Controlled environment studies demonstrated that *C. dematium*, *C. variabile* and *S. botryosum* can each infect and cause symptoms on spinach following leaf wetness durations of 1, 6, 24 or 48 h, with constant temperatures during the wet period of 5, 15, 20 or 25°C (Figure 1). For all three pathogens, leaf wetness duration was a key factor contributing to disease development; in general symptom incidence and severity was greater following 24 or 48 h leaf wetness compared to 1 or 6 h. Temperature appeared to be a

less important factor, although for Cladosporium leaf spot and anthracnose, 25°C was found to be least favourable for disease development.

Fungicide efficacy

In project year 1, 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. 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. 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 (cyprodinil + 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.

In a small-scale field experiment (project year 2), effective control of leaf spots was maintained until the harvest date, despite high inoculum pressure, using either two applications of Signum (9 and 14 days after sowing), or Signum (14 days) followed by Switch (21 days) (Figure 2). Signum has approval for use on spinach but has a 14 day harvest interval. Switch currently has no approval for spinach despite SOLAs on lettuce and outdoor herbs. Growers might consider whether a SOLA for this product would provide an opportunity for late season protection against leaf spots under high risk environmental conditions (if not precluded by MRL issues).



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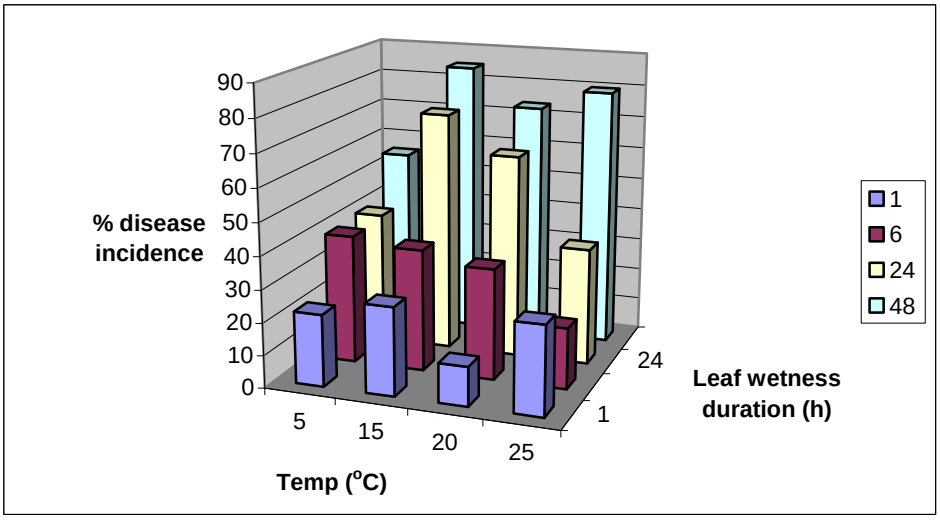

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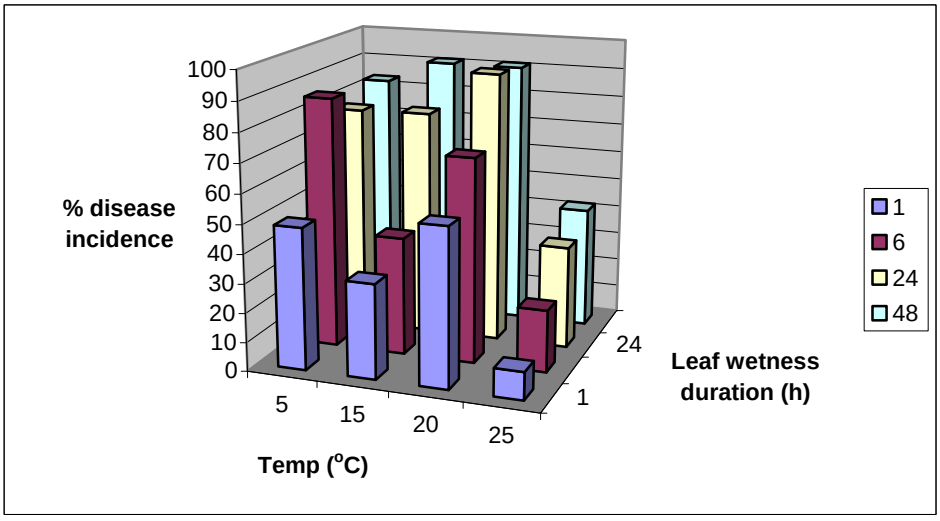


C

Figure 1. Effect of temperature and leaf wetness duration on spinach leaf spot incidence, 22 days after inoculation for A (*C. dematium*) and B (*S. botryosum*); 7 days after inoculation for C (*C. variabile*)

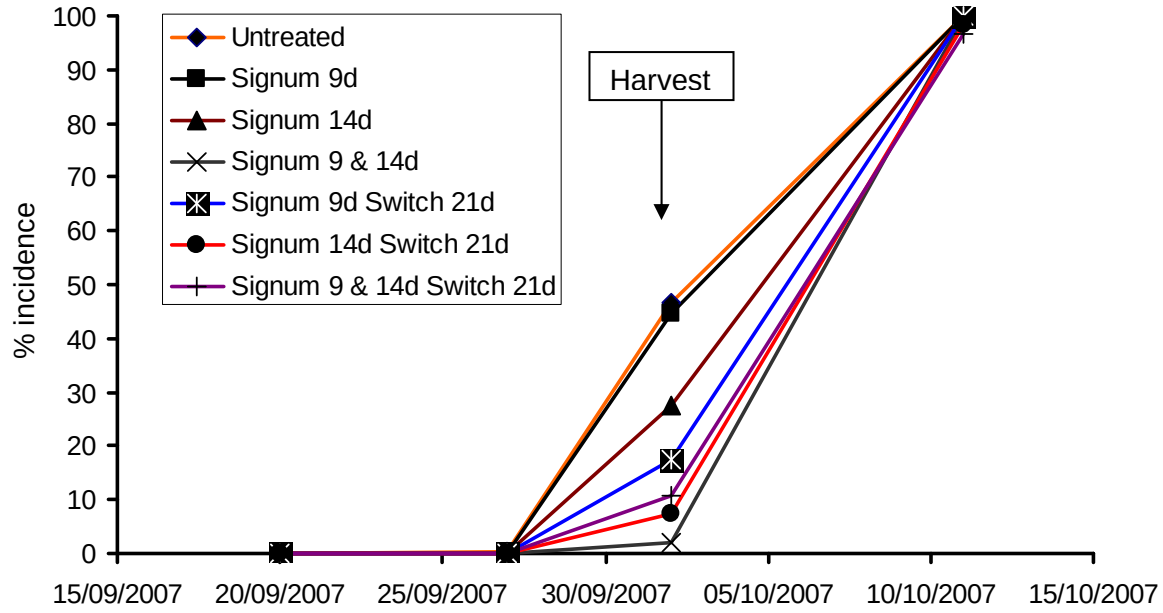


Figure 2. Effect of fungicide applications on incidence of leaf spots on spinach, 2007

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 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. Anthracnose and Cladosporium can survive on crop debris even when buried. Ensure that plants are properly killed (e.g. by herbicide application) between crops and seasons.
- When timing fungicide applications, be aware that crops are at high risk of developing leaf spots if leaf wetness duration exceeds 24 hours.
- Under high risk conditions for leaf spot development, 1 or 2 applications of Signum (boscalid + pyraclostrobin) (following the SOLA conditions of use) can provide effective 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* (Stemphylium leaf spot) 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* (Cladosporium leaf spot) 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 had not been established prior to this project.
- 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 2 of the project, focussing on sources of inoculum for leaf spot pathogens, environmental conditions favouring disease development and fungicide efficacy.

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). For production of conidia in certain experiments, it

was necessary to place cultures of the fungus under UV light to encourage spore production. 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. In situations where conidia were required, the fungus was cultured on V8 agar (200 ml V8 juice, 20 g agar powder, 3 g calcium carbonate in 1 L water) incubated at approximately 20°C under 12 h light/12 h dark, with plates left unsealed (L. du Toit, pers. comm.).

Sources of inoculum

The seed-borne nature of *S. botrysosum*, *C. dematium* and *C. variabile* on spinach seed has been confirmed (Hernandez-Perez & du Toit, 2006). In the previous annual report, levels of these pathogens in seed lots of varieties used for baby leaf spinach production in the UK were reported for the first time. Seed testing was continued in this project year, using seed supplied by three different seed houses. In addition, the importance of other sources of inoculum in baby leaf spinach production were investigated.

Seed

Objectives

- 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 from three seed houses
- To determine whether isolates of leaf spot pathogens from spinach seed are pathogenic to spinach.

Methods

100 g seed lots of spinach varieties used for baby leaf production in the UK were provided by three seed companies. A total of 11 untreated seed lots were tested in year 2. Four seed lots from Company A included two seed varieties previously tested in project year 1 (different seed lots) originating from Europe, and two seed lots obtained from New

Zealand. The four seed lots from Company B and three seed lots from Company C were of European origin.

For each seed lot, four composite samples of 6 g (approximately 500 seeds each) 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 lot). 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°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 14 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.

The mean incidence and standard deviation of seed-borne infection by individual pathogens was calculated for each seed batch.

Colonies identified as *C. variabile*, *S. botryosum* and *C. dematium* during the seed assay were transferred to agar to obtain pure cultures using the agar media described in Section 1.2. Representative isolates of pathogens obtained from seed tests in project year 1 and project year 2 were used in pathogenicity tests. For each isolate to be tested, three half trays of spinach (var. Lazio) with fifteen seeds per tray were sown using F2+S compost. When the seedlings had reached the 4 true-leaf stage, they were spray inoculated to run-off with a spore suspension of the fungal isolate (approximately 1 x 10^5 spores/ml) in SDW. Three half trays each of fifteen spinach seedlings were sprayed with SDW 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 around 7 and 14 days and typical lesions plated on to PDA+S or examined microscopically after damp incubation to confirm the causal organism.

Results and discussion

Colletotrichum dematium was not found in any of the 11 seed lots tested in project year 2 (Table 1). This was in agreement with seed test results from project year 1, when *C. dematium* was not recovered from any of 10 seed lots. 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 during this project suggests that other sources of inoculum may be of greater importance in outbreaks of spinach anthracnose in the UK.

Cladosporium variabile was not found in any of the eleven seed lots tested in project year 2 (Table 1). In project year 1, the pathogen was found at low levels (1.3% or less) in two out of the 10 seed lots tested. 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 our seed tests, non-pathogenic *Cladosporium* species were found on the seed but were classified as 'other fungi' rather than as *C. variabile*, based on conidial morphology and the absence of spiralling hyphae (Ellis, 1971). The presence of *C. variabile* in spinach seed lots (reported in Hernandez-Perez & du Toit, 2006 and from project year 1 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 contribute to outbreaks of Cladosporium leaf spot in the UK. However, given the relatively low frequency of occurrence across seed batches, and the 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 tested in project year 2 (Table 1). This was in agreement with

findings from project year 1 when nine out of 10 seed lots were infested with *S. botryosum* and/or *P. herbarum*. *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.* Where it occurred, the combined incidence of *S. botryosum* and *P. herbarum* on individual seed lots ranged from 0.8 to 29% in project year 1, and from 0.8 to 59% in project year 2. 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 nine of 11 seed lots examined in project year 2 (Table 1) and at least six of the 10 seed lots examined in project year 1. 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% in project year 1, and from 0.8 to 52% in project year 2. *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 some seed lots with relatively high infection levels.

Alternaria species were found on nine of 11 seed lots in project year 2 with incidence as high as 39%. The fungus was found in all 10 seed lots in project year 1 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.

The results demonstrated that the incidence of pathogens on seed of individual varieties can vary considerably with season (Table 1). For example, a seed lot of variety A2 harvested in 2006 had a low incidence of *Stemphylium botryosum* and a high incidence of *Verticillium dahliae*, while a seed lot of the same variety harvested in 2007 had high levels of *S. botryosum* and low levels of *V. dahliae.*

Representative isolates of *Stemphylium botryosum* (from project years 1 and 2) and *Cladosporium variabile* (from project year 1 only) from spinach seed were obtained in pure culture and were tested for their pathogenicity on spinach seedlings. Two isolates of *S. botryosum* (collected from seeds of different varieties) caused development of lesions typical of Stemphylium leaf spot on spinach leaves. When lesions were examined microscopically, spores of *S. botryosum* were observed in association with affected tissue, and the fungus was consistently re-isolated on agar. This result confirms that *S. botryosum* on spinach seed can be pathogenic to spinach (in agreement with results from Hernandez & Perez, 2006), although the risk of pathogen transfer from seed to seedlings (transmission rate) under field conditions has not been determined. *C. variabile* was found at a low incidence on seed in project years 1 and 2. A single isolate of *C. variabile* from seed did not cause symptom development on seedlings. This was in contrast to findings from Hernandez & Perez (2006) who demonstrated that *C. variabile* from spinach seed could be pathogenic to spinach. Pathogenicity testing of more isolates would be necessary to clarify this result.

Table 1. Incidence of leaf spot pathogens and other micro-organisms on untreated spinach seed of varieties commonly grown in the UK

Seed company / variety code	Seed origin	% seeds affected* (standard deviation)													
		*Stemphylium botryosum***		*Colletotrichum dematium*		*Cladosporium variabile*		*Verticillium dahliae*		*Alternaria* spp.		Other fungi		Bacteria	
A1 – 2006***	Europe	14.1	(9.5)	0.0	(0.0)	1.3	(2.8)	1.0	(2.1)	37.3	(11.6)	24.5	(15.0)	0.0	(0.0)
A1 – 2007	Europe	24.8	(12.9)	0.0	(0.0)	0.0	(0.0)	0.8	(1.8)	with 'other fungi'		75.5	(12.1)	0.0	(0.0)
A2 – 2006***	Europe	2.8	(3.8)	0.0	(0.0)	0.0	(0.0)	51.8	(10.7)	23.8	(10.8)	8.3	(6.7)	0.0	(0.0)
A2 – 2007	Europe	25.5	(14.6)	0.0	(0.0)	0.0	(0.0)	3.3	(4.1)	with 'other fungi'		70.8	(15.1)	0.0	(0.0)
A3	New Zealand	26.5	(12.3)	0.0	(0.0)	0.0	(0.0)	0.8	(1.8)	36.3	(18.2)	5.0	(6.7)	2.5	(4.4)
A4	New Zealand	30.5	(13.1)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	20.8	(10.3)	6.5	(6.9)	2.5	(5.3)
B1	Europe	1.8	(3.4)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	3.8	(8.1)	4.3	(5.2)	0.0	(0.0)
B2	Europe	5.0	(6.9)	0.0	(0.0)	0.0	(0.0)	3.5	(5.2)	0.0	(0.0)	1.0	(2.1)	0.0	(0.0)
B3	Europe	58.5	(14.6)	0.0	(0.0)	0.0	(0.0)	6.0	(5.8)	28.5	(15.4)	9.5	(7.8)	0.0	(0.0)
B4	Europe	0.8	(2.4)	0.0	(0.0)	0.0	(0.0)	2.3	(3.4)	0.0	(0.0)	0.8	(1.8)	0.0	(0.0)
C1	Europe	16.8	(10.3)	0.0	(0.0)	0.0	(0.0)	9.5	(7.1)	36.8	(14.4)	7.5	(7.7)	0.3	(1.1)

C2	Europe	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	51.0	(20.7)	1.0	(3.5)	1.3	(3.2)	0.0	(0.0)
C3	Europe	1.8	(2.9)	0.0	(0.0)	0.0	(0.0)	1.5	(3.3)	38.8	(17.2)	2.3	(3.8)	0.0	(0.0)

* Out of 400

** Combined incidence for both *S. botryosum* and its teleomorph *Pleospora herbarum*

*** Results from year 1 testing of same variety (but different seed lot); data included for comparison

Crop debris

Introduction and objective

In addition to seed-borne inoculum, alternative sources of inoculum for leaf spot pathogens were investigated. In the previous annual report, it was reported that *Colletotrichum dematium* (cause of spinach anthracnose) could survive on volunteer spinach plants from autumn through to the following February in a commercial field, following incomplete herbicide treatment. In project year 2, an experiment was done to determine the duration for which *C. dematium* and *C. variabile* could remain viable on spinach crop debris, under different environmental conditions. Plants infected with Stemphylium leaf spot were not available when this experiment was set up.

Methods

Spinach leaves (var. Lazio) artificially inoculated with *C. dematium* (April 2007) and showing typical symptoms of anthracnose were exposed to the following treatments:

	Debris position in soil	**Moisture treatment**
1	On soil surface	Dry
2	On soil surface	Wet
3	Buried in soil at 10-15 cm	Dry
4	Buried in soil at 10-15 cm	Wet

A plot comprised a gauze bag containing infected leaf debris. There were three replicate bags for each treatment and sampling time, to give a total of 48 bags. At each sampling time (3, 6, 9 and 12 months after inoculation), the presence or absence of *C. dematium* was determined in the laboratory.

Symptomatic leaf debris was collected from plant trays inoculated for a previous experiment, uprooting plants where necessary, and divided evenly into 48 samples. For treatments 1 and 2, a leaf debris sample was placed onto each of 24 nylon gauze pieces (approximately 10 cm^2). The gauze was gathered into 'bags' using an elastic band and

a plastic label with treatment and replicate number was attached. For treatments 3 and 4, each leaf debris sample was mixed with compost from the original seed trays and placed onto each of 24 gauze pieces then made into labelled bags.

24 pots (12 cm diameter) were filled with field soil. For treatments 3 and 4, bags were buried to 10-15 cm depth, with 1 bag per pot. Bags for treatment 1 were placed on the surface of pots containing bags for treatment 3. Bags for treatment 2 were placed on the surface of pots containing bags for treatment 4.

The pots were maintained in a glasshouse. Pots for treatments 1 and 3 were kept dry (no watering). Pots for treatments 2 and 4 were hand watered as necessary to maintain moist but not water-logged soil. At each sampling time, the appropriate bags (3 per treatment) were collected from the pots. For each bag, debris was examined microscopically to check for the presence of setae indicative of *C. dematium*. In addition, debris pieces were surface sterilised in 90% ethanol (10 sec), dried on filter paper, then plated onto agar (PDA+S). The proportion of plated debris pieces from which *C. dematium* developed was assessed after 7 and 14 days. For sampling at 3 and 6 months, 5 debris pieces per bag were used; for sampling at 9 and 12 months, 15 debris pieces per bag were used.

A separate experiment was set-up using the same procedure as described above but using leaves of spinach var. Lazio that had been artificially inoculated with *Cladosporium variabile* (May 2007) and showing typical and severe symptoms of Cladosporium leaf spot.

Results and discussion

The results (Tables 2 and 3) demonstrated that *Colletotrichum dematium* and *Cladosporium variabile* can survive under certain conditions on spinach crop debris in soil for a period of at least 12 months.

At each sampling time, setae (black hair-like structures) were observed microscopically on debris pieces from all treatments demonstrating the presence of *C. dematium* (spinach anthracnose). Despite a low incidence of retrieval at 6 months, the pathogen was subsequently recovered from three of the treatments at 9 months and all treatments at 12 months. There was a trend for a higher incidence of pathogen survival at the soil surface under moist conditions. The results indicate that this pathogen could persist between growing seasons on crop debris, either at the surface or following incorporation to a depth of 15 cm, irrespective of moisture conditions. Growers should be aware of the need for thorough crop destruction (e.g. by herbicide treatment) to eliminate this pathogen between growing seasons.

For *C. variabile* (Cladosporium leaf spot), there was no isolation of the fungus at 3 months (reason not clear) but there was a high incidence of isolation at 6 months. Subsequently, at 9 and 12 months *C. variabile* was isolated only from dry material on the soil surface. It appears that in contrast to *C. dematium*, *C. variabile* was unable to remain viable once debris became more decomposed. The results suggest that debris infested with *C. variabile* could be a source of inoculum for disease outbreaks for a period of around 6 months. Under normal field conditions (fluctuating wet and dry), *C. variabile* is less likely to survive for longer periods, even when on the soil surface.

Table 2. Effect of debris position and moisture in soil on survival of *Colletotrichum dematium* on spinach debris

Debris position in soil	Moisture treatment	Mean % debris pieces with viable *C. dematium* at intervals after inoculation*			
		3 months	6 months	9 months	12 months
On soil surface	Dry	100	0	9	9
On soil	Wet	93	0	53	24

surface					
Buried in soil at 10-15 cm	Dry	0	13	0	7
Buried in soil at 10-15 cm	Wet	0	0	4	2

*Mean for 3 reps of five pieces each (3 & 6 month samples), and 15 pieces each (9 & 12 months sample)

Table 3. Effect of debris position and moisture in soil on survival of *Cladosporium variabile* on spinach debris

Debris position in soil	Moisture treatment	Mean % debris pieces with viable *C. variabile* at intervals after inoculation*			
		3 months	6 months	9 months	12 months
On soil surface	Dry	0	67	9	27
On soil surface	Wet	0	33	0	0
Buried in soil at 10-15 cm	Dry	0	40	0	0
Buried in soil at 10-15 cm	Wet	0	contents disintegrated	0	0

*Mean for 3 reps of five pieces each (3 & 6 month samples), and 15 pieces each (9 & 12 months sample)

Fungicide efficacy

Objectives

In 2006, pot experiments were done to determine the efficacy of a range of fungicides for control of spinach leaf spots, when applied at different timings in relation to infection events (Year 1 Annual report). In 2007, a small-scale field experiment was done, using products currently approved for use on spinach, to further investigate optimum product timing, taking account of growing season duration (e.g. 28 days) and specified harvest intervals.

Methods

The experiment comprised a randomised complete block design with four replicate blocks. There were six fungicide programmes, plus an untreated control (seven treatments) (Table 4).

Table 4. Fungicide programmes evaluated against spinach leaf spot diseases (Sept, 2007)

	Fungicide	Time of application (days after planting)		
	Treatment	9	14	21
1	Untreated control	-	-	-
2	Signum only (1)	Signum	-	-
3	Signum only (2)	-	Signum	-
4	Signum only (3)	Signum	Signum	-
5	Signum/Switch (1)	Signum	-	Switch
6	Signum/Switch (2)	-	Signum	Switch
7	Signum/Switch (3)	Signum	Signum	Switch

Fungicides were applied 9, 14 and 21 days after planting, according to the treatment list in Table 4. Products were applied in 1000 L water/ha at 2 Bar pressure using an Oxford precision sprayer with single nozzle. Product rates are shown below:

Product	**Active ingredient**	**Product rate**
Signum	boscalid + pyraclostrobin	1.5 kg/ha

Switch cyprodinil + fludioxonil 0.8 kg/ha

Notes:

Signum – SOLA 2378/05

Switch – Administrative Experimental Approval (use rate from SOLA 2079/07 on lettuce)

Plots comprised seven rows (1.5 m length) spaced at 7 cm, with plant spacing of approximately 2.5 cm (to reflect commercial practice). The outer two rows and end four plants of each plot were used as guards and the inner five rows as experimental plants. There was a spacing of 0.3 m between plots along the row (but planted with spinach) and pathways of at least 1 m between blocks. Irrigation pipes with four evenly spaced uprights for misting were laid between blocks 1 and 2, and between blocks 3 and 4. The experimental area was contained within fencing to prevent rabbit damage. Experimental layout is shown in Appendix 1.

The experiment was sited on an area of land at ADAS Arthur Rickwood that had previously been covered with mypex for at least 7 years. The trial area was rotavated then re-rotavated once seedling germination had occurred. Following soil sampling and analysis, a base dressing of nitrogen, phosphate and potassium was incorporated during the second rotavation at 50 kg/ha, 150 kg/ha and 175 kg/ha, respectively (N. MacDonald / I. Gillott, pers. comm.). Residual herbicides were considered inappropriate for the trial area due to the high percentage organic matter content and were not used.

The trial area was lightly raked to ensure a smooth seed bed and marked out as shown in the experimental plan. Seeds (var. Lazio) were sown at a depth of approx 1.5 cm and a spacing of approx 2.5 cm along the row. The sowing date was 4 September 2007. Irrigation was applied for 15 min after sowing to achieve a moist seed bed.

Three artificial inoculations were done to reflect what might occur under field conditions. Inoculum for the field experiment was prepared as follows. Fifty plate cultures of each of *C. dematium*, *C. variabile* and *S. botryosum* were prepared using the techniques described in Section 1.2. For the 1st artificial inoculation (9 days after planting), the plates were used to prepare a spore suspension of all three pathogens. For each pathogen, a sterile

loop was used to scrape spores from the surface of the cultures into distilled water. The spore suspension was filtered through muslin. The total volume applied to the experiment area using a hand mister was 1925 ml containing 4 x 10^4 spores/ml of *C. dematium* and 5.6 x 10^4 spores/ml of *C. variabile*. Spores of *S. botryosum* had not developed on the culture plates. Overhead mist irrigation was applied for 10 minute periods at intervals over the next 24 hours to ensure continuous leaf wetness. This inoculation was done on the same day as the first fungicide application, with the fungicide applied in the morning, and inoculation in the afternoon.

14 days after planting, an additional source of inoculum, dried leaf material infected with *C. dematium* and *C. variabile* (remaining from a previous trial) was introduced to the experimental area. Since plates of *S. botryosum* were still not sporulating, mycelium on agar from the Stemphylium cultures was cut into squares (approx 2 cm^2) and mixed with the leaf debris. The debris mixture was laid on the ground (slightly submerged in soil), at two positions adjacent to each plot. Mist irrigation was applied at intervals to ensure continuous leaf wetness for at least 12 h after inoculation. The 2nd inoculation was done on the day after the 2nd fungicide application.

21 days after planting, a 3rd inoculation was done using spores of *C. dematium* and *C. variabile*. A total of 1.4 L spore suspension was applied using a hand mister, containing 4.7 x 10^4 spores *C. dematium* and 5.3 x 10^4 spores *C. variabile*. Infested leaf debris (both pathogens) remaining from previous experiments was also chopped up and added to the plots. The plots were covered with polythene for 48 h to maintain leaf wetness. The 3rd inoculation was done on the day of the 3rd fungicide application, with the fungicide applied in the morning, and inoculation in the afternoon.

On days when there was no rainfall, overhead mist irrigation was applied to ensure that the soil remained moist without being waterlogged. Weed and pest control was not required during the experiment.

Disease incidence and severity was assessed 16, 21, 28 and 37 days after planting. Under commercial conditions, the crop would have been harvested at 28 days at this time of the year. For each plot, 20 consecutive plants were assessed in each of the middle five rows for the presence or absence of leaf spots. For disease severity, the percentage leaf area affected on the 20 plants per row was estimated, giving five disease severity scores per plot.

At each assessment date, plants were checked for any phytotoxic symptoms or growth benefits (e.g. greening) from the treatments applied. Lesions representative of different leaf symptom types were plated on PDA+S (after surface sterilising in 1% sodium hypochlorite for 30 sec, followed by two rinses in sterile distilled water) and incubated at approximately 20°C to confirm the causal organisms. Data for percentage disease incidence and severity was analysed by ANOVA in Genstat.

Summary of field operations:

Date:	**Days after planting:**	**Task:**
04.09.07	1	Sow experiment
13.09.07	9	Spray application 1
13.09.07	9	Inoculation 1
18.09.07	14	Spray application 2
19.09.07	15	Inoculation 2
20.09.07	16	Assessment 1
25.09.07	21	Spray application 3
25.09.07	21	Inoculation 3
27.09.07	23	Assessment 2
02.10.07	28	Assessment 3 (Harvest date)
11.10.07	37	Assessment 4

Results and discussion

There were no phytotoxic or beneficial effects of any of the treatments applied in terms of plant growth.

At the end of the experiment, lesions on leaves from all treatments were found to be due consistently to *C. dematium* (anthracnose) and *C. variabile* (Cladosporium leaf spot). Lesions due to *S. botryosum* were not found (even in the untreated control), indicating that inoculation with this pathogen had not been successful.

No disease symptoms were observed on the crop until 21 days after planting, when one plant with leaf spot symptoms was observed in the untreated control. At 28 days after planting (commercial harvest date), disease incidence had increased substantially (Figure 1) up to 47% of plants affected in the untreated control. Disease severity remained low (<1% leaf area affected for all treatments) although in a commercial situation even low levels of blemishes on baby-leaf spinach can cause crop rejection. There was a significant effect of treatment on disease incidence at the time when the crop would have been harvested. Disease incidence following a single treatment with Signum at 9 days or at 14 days after planting was no different from the untreated control (Table 5). The remaining treatments significantly reduced leaf spot incidence compared to the untreated control. In terms of reducing disease incidence and severity at harvest, there was no benefit in applying three sprays instead of two, with programmes of Signum at 9 and 14 days, or of Signum (14 days) then Switch (21 days), providing equivalent control. The crop was left in the ground for a further 9 days to determine the persistence of the final spray application. At the final assessment, incidence had increased to over 95% for all treatments but there was a significant effect of the treatment on disease severity. Again, a single treatment with Signum at 9 or 14 days did not reduce disease severity compared with the untreated control. All other treatments significantly reduced disease severity, and there was no statistical difference between these treatments.

Leaf spots on spinach have to be kept to a minimum because of retailer pressure for blemish-free produce. Timing fungicides to achieve effective leaf spot control on baby leaf spinach can be problematic because of the short growing season, required harvest intervals for approved fungicides and difficulty in predicting when infection might occur. In this experiment, even under high inoculum pressure (indicated by high disease incidence in the untreated control), effective disease control was maintained up until the harvest date using Signum applied early and mid-way through the season, adhering to the 14-day harvest interval for this product. A single application of Signum at 9 days after planting did not contribute greatly to disease control. This suggests that conditions for infection were not ideal following the first inoculation (immediately after the first fungicide application) and that it was the 2nd and 3rd inoculations that resulted in observed disease development. A

spray at 9 days after planting could still be beneficial in field production, if conditions have been conducive for disease development at the time of emergence. Programmes in which one or two Signum applications were followed by an application of Switch (7-day harvest interval) were effective in reducing leaf spot incidence at harvest. In addition, an assessment of disease severity after the estimated harvest date demonstrated that there was a trend for lower disease severity on treatments that had received Signum at 14 days then Switch at 21 days.

It should be noted that Switch is not currently approved for use on spinach but has SOLAs for other salad crops such as lettuce and outdoor herbs.

Table 5. Effect of fungicide programmes on the incidence and severity of spinach leaf spots in an inoculated experiment (Cambridgeshire, September 2007)

Fungicide programme	% incidence (I) and severity (S) at days after planting							
	16		21		28*		37	
	I	S	I	S	I	S	I	S
Untreated control	0.0	0.0	0.25	<0.1	46.5	0.2	100.0	16.7
Signum (9 d)	0.0	0.0	0.0	0.0	44.5	0.1	100.0	18.5
Signum (14 d)	0.0	0.0	0.0	0.0	27.8	0.1	100.0	9.8
Signum (9 & 14 d)	0.0	0.0	0.0	0.0	2.0	<0.1	99.8	4.8
Signum (9 d) Switch (21 d)	0.0	0.0	0.0	0.0	17.5	<0.1	100.0	3.8
Signum (14 d) Switch (21 d)	0.0	0.0	0.0	0.0	7.3	<0.1	98.2	0.5
Signum (9 & 14 d) Switch (21 d)	0.0	0.0	0.0	0.0	10.8	<0.1	96.8	0.4
D.f.	-	-	-	-	18	-	18	18
F. probability					0.003		0.338	0.001
LSD					23.3		3.4	8.8

*Equivalent to harvest date

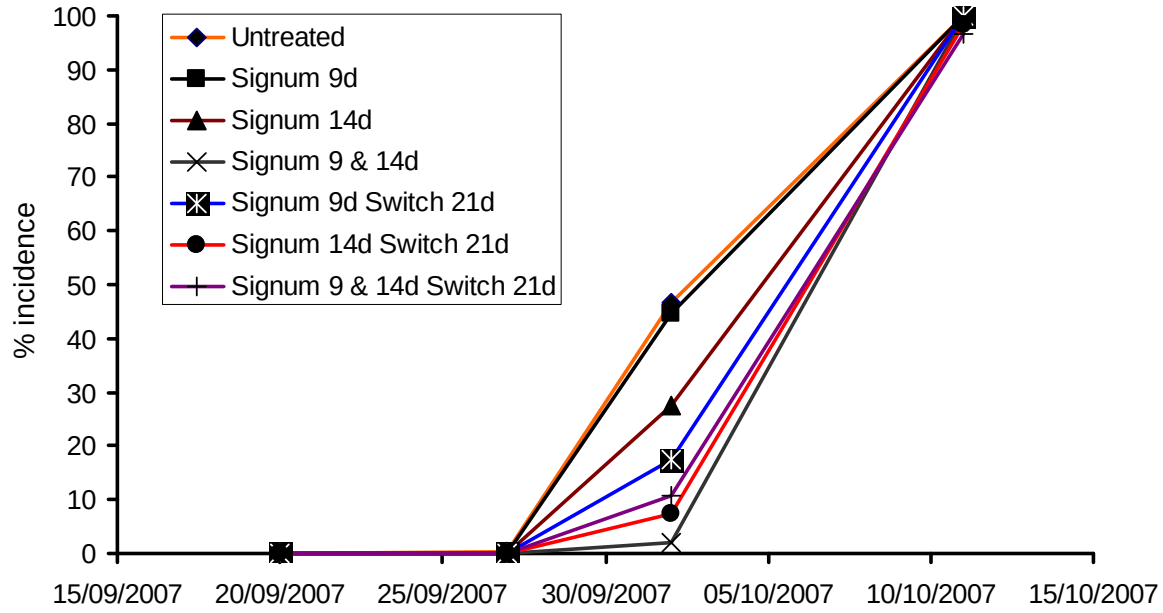


Figure 1. Effect of fungicide applications on incidence of leaf spots on spinach

Effect of environmental conditions on the development of spinach leaf spots

Objective

To determine the effect of temperature and leaf wetness on the development of spinach leaf spot symptoms after artificial inoculation with each of three pathogens:

- *Colletotrichum dematium*
- *Cladosporium variabile*
- *Stemphylium botryosum*

Methods

Experimental design

Spinach plants inoculated with one of three pathogens (*C. dematium*, *C. variabile* and *S. botryosum*), were incubated at the following temperatures: 5°C, 15°C, 20°C and 25°C. For each temperature treatment, four leaf wetness durations were tested (1 h, 6 h, 24 h and 48 h).

The effects of environmental conditions were tested simultaneously for plants inoculated with one of the three pathogens but results for each of the three pathogens were considered

separately. The experiment tested the effects of four temperatures and four leaf wetness durations in a factorial design, with three replicate blocks (over time) for each treatment combination. A plot comprised a seed tray sown with 15 plants.

The experiment was done using two controlled environment (CE) cabinets each set at a different temperature (see below). Each temperature was tested three times. For each run, a single cabinet contained for each of the three pathogens, one seed tray (of 15 plants) for each of the four leaf wetness durations, to give 12 trays in total. Trays inoculated with the different pathogens were separated within the cabinet by vertical plastic barriers, to prevent cross contamination. For each pathogen, the position of trays for the different leaf wetness durations was randomised.

		Cabinet 1	Cabinet 2
Week 1	Run 1 & 2 (mon-weds)	5°C	25°C
	Run 3 & 4 (weds-fri)	20°C	15°C
Week 2	Run 5 & 6 (mon-weds)	20°C	5°C
	Run 7 & 8 (weds-fri)	15°C	25°C
Week 3	Run 9 & 10 (mon-weds)	15°C	5°C
	Run 11 & 12 (weds-fri)	25°C	20°C

For each run, after the appropriate leaf wetness duration, plants were air dried with an electric fan then moved to a glasshouse bench on capillary matting to monitor symptom development. Plants inoculated with different pathogens were positioned on different areas of the bench to avoid cross-infection. The position of trays from different runs was randomised on the glasshouse bench.

Sowing of test plants was staggered to ensure that plants of similar age were used for each run of the experiment. At each sowing (twice a week for three weeks), an extra tray of 15 plants was sown. These plants remained uninoculated and were transferred directly to the glasshouse at the start of each pair of runs rather than being placed in a CE cabinet. They were positioned away from the inoculated plants and served as a check

for development of foliar disease due to seed-borne inoculum rather than due to artificial inoculum.

As temperatures were tested over time, their effect could have been confounded with that of inoculum. To minimise confounding, percentage spore germination for each pathogen was determined by microscopic examination of inoculum plated onto agar, to ensure that spore viability remained uniformly high for all runs.

Production of plants

At each sowing date (twice per week for 3 weeks), 27 half-size seed trays were filled with F2+S compost and sown with 15 pelleted spinach seeds var. Lazio (three rows of five). The trays were labelled with the sowing date and maintained at 20°C in a glasshouse on capillary matting. The trays were overhead watered to maintain moist but not waterlogged compost for 3 weeks (until approximately 2-3 true leaf stage). Each sowing provided 12 trays for each of two cabinets, one tray as an uninoculated control and two trays spare, in case of poor germination.

Production of sporulating cultures

Sporulating cultures of *S. botryosum*, *C. dematium* and *C. variabile* were produced ready for each run of the experiment as described in Section 1.2.

Inoculation

At the start of each temperature run, a spore suspension of each pathogen was prepared by pouring approximately 5 ml sterile distilled water onto each culture and using a sterile loop to dislodge the spores. The spore suspension was filtered through muslin and the spore concentration checked using a haemocytometer and high power microscope (aiming for 1×10^5 spores/ml). The appropriate trays of spinach were sprayed with the spore suspension to the point of run-off using a hand-held mister.

Plants to be inoculated with different pathogens were separated during the inoculation procedure to avoid cross-contamination while spraying to run-off. A different mister was

used for each pathogen, and the bottle rinsed with water, then 90% ethanol before and after use.

Testing spore viability

At each inoculation, a sterile loop was used to streak the spore suspension from each pathogen onto a plate of PDA+S. The plates were incubated for 16 h at approx 20°C before checking percentage spore germination on 3 lots of 100 spores.

CE cabinet set-up

Each cabinet was set to the required temperature at least 1 hour before required with a 12 h day / 12 h night light regime and 0% RH. Two plastic vertical barriers were used to separate plants inoculated with the three different pathogens. Once inoculated plants were placed in the cabinets a misting unit (1.2 L water/h) was used to maintain continuous leaf wetness for 48 h.

Leaf wetness durations

Uninoculated plant trays were labelled and maintained on the glasshouse bench. Trays of plants receiving a 1 h leaf wetness treatment were inoculated then dried with an electric fan on a laboratory bench (rather than being placed in the controlled environment cabinet) then returned to the glasshouse bench. Plants for the remaining leaf wetness treatments were placed in the CE cabinets after inoculation and left there until the end of the required leaf wetness period (6, 24 or 48 h). After this period, plants were air dried and transferred to the glasshouse bench.

Plant maintenance in glasshouse

The plants were maintained in the glasshouse on damp capillary matting at 20°C. There was no overhead watering.

Assessments

For each run, each plot (15 plants) was assessed 8, 15 and 22 days after inoculation for:

- Number of plants emerged per tray
- Incidence of leaf spot symptoms (number of plants per tray affected)
- Severity of leaf spot symptoms (percentage leaf area affected for each of four plants in the centre of each tray)

For each pathogen, typical lesions were checked under the microscope and by plating onto PDA+S (following surface sterilising in 90% ethanol for 10 sec and drying on filter paper), to confirm the causal organism.

The effects of temperature and leaf wetness duration on disease incidence and severity was analysed using ANOVA in Genstat.

Results and discussion

From microscopic examination of damp incubated leaves inoculated with the different pathogens, the causal organisms of lesions were confirmed as those which had been used for the original inoculations.

Colletotrichum dematium

(see Figure 2A; Appendix 2)

Disease development was observed on all treatments by the end of the experiment irrespective of temperature or leaf wetness duration applied. There was a significant effect of leaf wetness on percentage incidence of anthracnose at each of the three assessments ($P<0.001$). At the first assessment, incidence approximately doubled with increasing leaf wetness durations. At the 2nd and 3rd assessments, incidence was higher following leaf wetness duration of 24 or 48 h compared to 1 or 6 h. There was a similar effect on disease severity at the 3rd assessment, although mean severity remained low (6% or less). At the 1st assessment, disease incidence was higher at 20°C than at 25 or 5°C, but there were no significant effects due to temperature at later assessments, or significant interaction effects.

Stemphylium botryosum

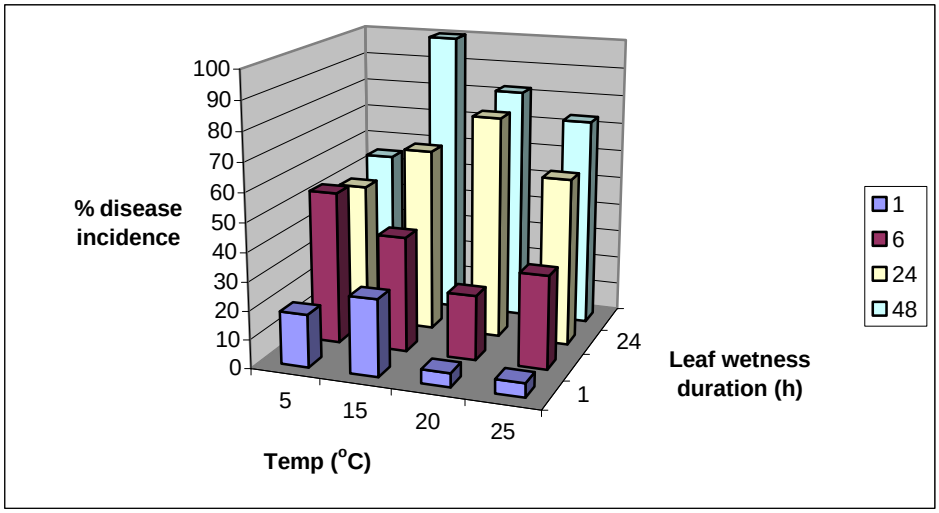
(See Figure 2B; Appendix 2)

Stemphylium leaf spots were recorded for all treatments. There were no significant effects due to temperature or interaction effects. For disease incidence and severity at the 1st assessment and severity at the 2nd assessment, there were no significant treatment effects. At the 2nd assessment, disease incidence was approximately double following a leaf wetness duration of 48 h ($P<0.05$) compared with durations of 1 or 6 h. At the 3rd assessment, there were treatment effects due to leaf wetness duration, with incidence highest following 48 h leaf wetness, and severity highest following 24 or 48 h leaf wetness.

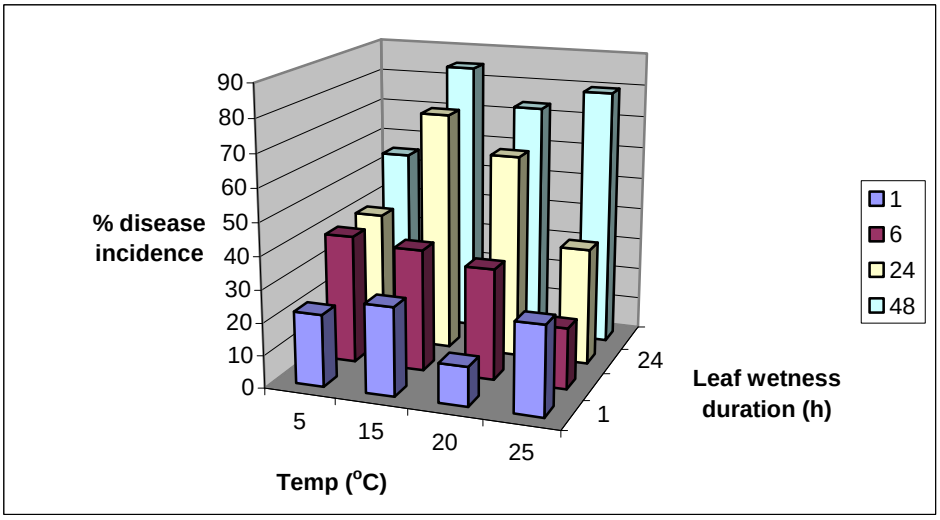
Cladosporium variabile

(see Figure 2C; Appendix 2)

Cladosporium leaf spot developed rapidly for all treatments with a mean of over 90% incidence for all treatments by the 2nd assessment. At the 1st assessment there were significant effects of temperature ($P<0.001$) and leaf wetness duration ($P=0.001$). Disease incidence was higher after 24 or 48 h leaf wetness, and after temperatures of 5, 15 or 20°C compared with 25°C. Disease severity was low at the 1st assessment (<0.5%) and increased to a maximum of 11% by the 3rd assessment. At the 3rd assessment, disease severity was higher following leaf wetness durations of 24 or 48 h. A temperature of 25°C was least conducive to disease development, and 20°C was the most favourable.



A



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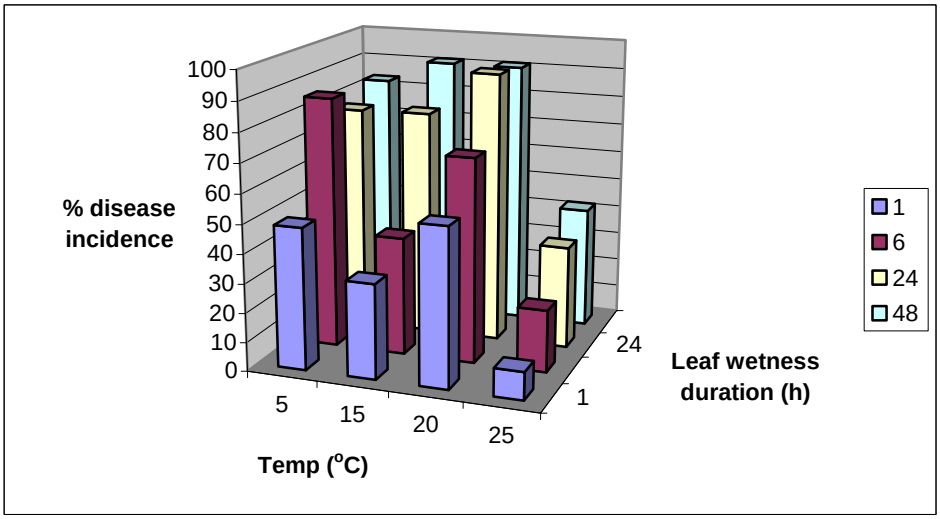

C

Figure 2. Effect of temperature and leaf wetness duration on spinach leaf spot incidence, 22 days after inoculation for A (*C. dematium*) and B (*S. botryosum*); 7 days after inoculation for C (*C. variabile*)

Project conclusions

Leaf spot pathogens

Studies in project FV 268 and subsequently in FV 268a have demonstrated that leaf spots on baby leaf spinach in the UK can be caused by *Colletotrichum dematium* (anthracnose), *Cladosporium variabile* (Cladosporium leaf spot) and *Stemphylium botryosum* (Stemphylium leaf spot). Attempts to produce sporulating cultures of *S. botryosum* have been only partially successful, despite testing a range of techniques used by other researchers. For this reason, results on varietal susceptibility and fungicidal control (experiments requiring artificial inoculation using sporulating cultures) have been limited to anthracnose and Cladosporium leaf spot only. Experiments on seed-borne inoculum and effects of environmental conditions on leaf spot development have been inclusive of all three pathogens.

Sources of inoculum

Twenty-one spinach seed lots (17 varieties) were tested during the project, covering two seed harvest seasons, with 19 lots produced in Northern Europe and 2 lots produced in New Zealand. The seed lots were provided by three seed companies. *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 19 out of 21 seed lots, with percentage incidence ranging from 0.8 to 59%. Stemphylium isolates from seed were pathogenic to spinach. *C. variabile* affected 2 out of 21 seed lots (incidences of 0.3 and 1.3%), and isolate pathogenicity was not demonstrated. *Colletotrichum dematium* (spinach anthracnose) was not detected in any of the seed lots. *Verticillium dahliae* was also detected in 15 seed lots tested (0.3 – 52% incidence). 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.

Infection by *Colletotrichum dematium* (producing viable conidia) was confirmed on overwintering spinach volunteers at a commercial holding, demonstrating a potential source of inoculum for spinach anthracnose if crops are not thoroughly destroyed after harvest.

C. dematium (anthracnose) and *C. variabile* (Cladosporium leaf spot) survived under certain conditions on spinach crop debris for a period of at least 12 months. *C. dematium* survived both on the soil surface and to a depth of 15 cm, under both wet and dry soil conditions. *C. variabile* remained viable for up to 6 months on the soil surface and buried; after this period it was only recovered from dry debris on the soil surface (unlikely to occur under normal field conditions)

From experimental results it is concluded that the following sources of inoculum are most likely to be contributing to outbreaks of individual leaf spot diseases on UK baby-leaf spinach:

- *Stemphylium botryosum* (Stemphylium leaf spot) – seed
- *Cladosporium variabile* (Cladosporium leaf spot) – seed and infested debris (up to 6 months)
- *Colletotrichum dematium* (anthracnose) – infested debris (at least 12 months) and volunteer plants

Fungicide efficacy

Of seven products tested in inoculated pot trials in project year 1, 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.

In a small-scale field experiment (project year 2), effective control of leaf spots was maintained until the harvest date, despite high inoculum pressure, using either two

applications of Signum (9 and 14 days after sowing), or Signum (14 days) followed by Switch (21 days). Signum has approval for use on spinach but has a 14 day harvest interval. Switch currently has no approval for spinach despite SOLAs on lettuce and outdoor herbs. Growers might consider whether a SOLA for this product would provide an opportunity for late season protection against leaf spots under high risk environmental conditions (if not precluded by MRL issues).

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.

Effect of environmental conditions

Controlled environment studies demonstrated that *C. dematium*, *C. variabile* and *S. botryosum* can each infect and cause symptoms on spinach following leaf wetness durations of 1, 6, 24 or 48 h, with constant temperatures during the wet period of 5, 15, 20 or 25°C. For all three pathogens, leaf wetness duration was an important factor contributing to disease development; in general symptom incidence and severity was greater following 24 or 48 h leaf wetness compared to 1 or 6 h. Temperature appeared to be less of a key factor, although for Cladosporium leaf spot and anthracnose, 25°C was found to be least favourable for disease development.

Technology transfer (project year 2)

- Telephone and email responses to growers requesting information on sample diagnosis in 2007-2008
- Diagnosis of spinach leaf spot samples sent by growers.

- Green KR. 2008. Spinach leaf spots and their management. HDC Factsheet (update)t. East Malling Kent: Horticultural Development Council (*in preparation*)
- Project review meeting, 25 May 2007, Langmeads Farm.
- Summary of results presented to the SPGA, Boxworth, Cambs, February 2008
- Presentation to British Leafy Salads Conference, invited for September 2008

References

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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).

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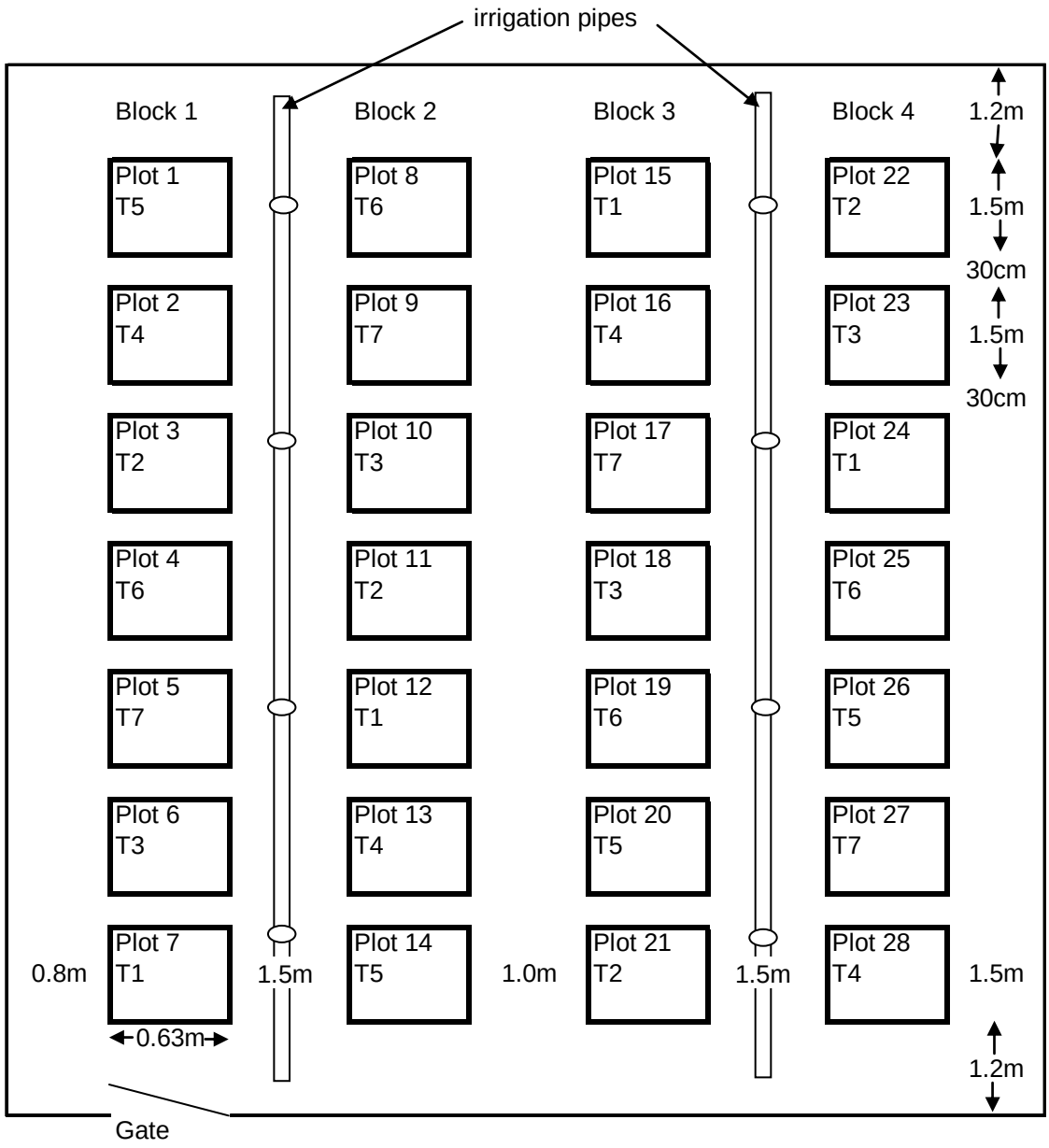
APPENDIX 1

Experiment layout: fungicide efficacy 2007



Each plot has 7 rows

APPENDIX 2.

ANOVAs and means for effect of temperature and leaf wetness duration on the incidence and severity of spinach leaf spots

Colletotrichum dematium

Variate: %_incidence_Assessment3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	11494.0	5747.0	9.59	<.001
Temp	3	1747.2	582.4	0.97	0.419
Leafwetness	3	28503.5	9501.2	15.85	<.001
Temp.Leafwetness	9	5258.1	584.2	0.97	0.480
Residual	30	17987.2	599.6		
Total	47	64989.9			

Tables of means

Variate: %_incidence_Assessment3

Grand mean 47.7

Rep	1	2	3
	25.8	58.9	58.5

Temp	5	15	20	25
	44.0	57.8	46.8	42.3

Leafwetness	1	6	24	48
	13.7	37.2	62.5	77.5

Temp	Leafwetness	1	6	24	48
5		18.4	53.3	49.4	54.8
15		26.7	40.2	64.3	100.0
20		4.8	22.7	77.8	82.1
25		4.9	32.5	58.6	73.3

Least significant differences of means (5% level)

Table	Rep	Temp	Leafwetness	Temp Leafwetness
rep.	16	12	12	3
d.f.	30	30	30	30
l.s.d.	17.68	20.42	20.42	40.83

Variate: Mean_severity_Assessment3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	66.752	33.376	6.38	0.005
Temp	3	41.006	13.669	2.61	0.069
Leafwetness	3	94.101	31.367	6.00	0.002
Temp.Leafwetness	9	58.029	6.448	1.23	0.313
Residual	30	156.839	5.228		
Total	47	416.727			

Variate: Mean_severity_Assessment3

Grand mean 1.60

Rep	1	2	3
	0.18	3.06	1.55

Temp	5	15	20	25
	0.44	1.99	2.89	1.07

Leafwetness	1	6	24	48
	0.10	0.41	2.42	3.47

Temp	Leafwetness	1	6	24	48
5		0.01	0.65	0.27	0.85
15		0.32	0.62	2.00	5.01
20		0.05	0.01	5.22	6.26
25		0.01	0.35	2.17	1.76

Least significant differences of means (5% level)

Table	Rep	Temp	Leafwetness	Temp Leafwetness
rep.	16	12	12	3
d.f.	30	30	30	30
l.s.d.	1.651	1.906	1.906	3.813

Stemphylium botryosum

Analysis of variance

Variate: %_incidence_Ass3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	4711.7	2355.9	4.17	0.025
Temp	3	2058.7	686.2	1.21	0.322

Leafwetness	3	18507.8	6169.3	10.92	<.001
Temp.Leafwetness	9	3659.3	406.6	0.72	0.687
Residual	30	16952.9	565.1		
Total	47	45890.3			

Tables of means

Variate: %_incidence_Ass3

Grand mean 45.3

Rep	1	2	3
	43.5	34.2	58.2

Temp	5	15	20	25
	39.3	55.9	45.6	40.4

Leafwetness	1	6	24	48
	22.2	32.5	53.3	73.2

Temp	Leafwetness	1	6	24	48
5		22.2	39.6	40.5	55.1
15		27.1	37.6	74.0	84.8
20		12.1	34.1	62.7	73.4
25		27.3	18.7	36.0	79.6

Least significant differences of means (5% level)

Table	Rep	Temp	Leafwetness	Temp Leafwetness
rep.	16	12	12	3
d.f.	30	30	30	30
l.s.d.	17.16	19.82	19.82	39.64

Analysis of variance

Variate: Mean_Severity_Ass3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	4.4053	2.2027	3.12	0.059
Temp	3	2.4584	0.8195	1.16	0.341
Leafwetness	3	11.3638	3.7879	5.37	0.004
Temp.Leafwetness	9	4.5019	0.5002	0.71	0.696
Residual	30	21.1651	0.7055		
Total	47	43.8945			

Tables of means

Variate: Mean_Severity_Ass3

Grand mean 0.60

Rep	1	2	3
	0.34	0.43	1.02

Temp	5	15	20	25
	0.33	0.82	0.82	0.42

Leafwetness	1	6	24	48
	0.07	0.19	0.89	1.24

Temp	Leafwetness	1	6	24	48
5		0.07	0.33	0.43	0.47
15		0.12	0.22	1.10	1.83
20		0.02	0.15	1.73	1.40
25		0.07	0.06	0.30	1.25

Least significant differences of means (5% level)

Table	Rep	Temp	Leafwetness	Temp Leafwetness
rep.	16	12	12	3
d.f.	30	30	30	30
l.s.d.	0.606	0.700	0.700	1.401

Cladosporium variabile

Variate: %_incidence_Assessment1

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Rep	2	1903.3	951.6	1.68	0.204
Temp	3	18818.5	6272.8	11.07	<.001
Leafwetness	3	11984.7	3994.9	7.05	0.001
Temp.Leafwetness	9	2904.4	322.7	0.57	0.811
Residual	29 (1)	16440.0	566.9		
Total	46 (1)	52043.6			

Tables of means

Variate: %_incidence_Assessment1

Grand mean 59.6

Rep	1	2	3
	52.4	58.6	67.7

Temp	5	15	20	25
	73.8	60.5	77.0	27.0

Leafwetness	1	6	24	48
	36.2	54.5	70.8	76.9

Temp	Leafwetness	1	6	24	48
5		48.6	86.0	77.3	83.3
15		32.5	40.5	77.8	91.1
20		54.1	70.0	92.9	91.1
25		9.5	21.3	35.3	41.9

Least significant differences of means (5% level)

Table	Rep	Temp	Leafwetness	Temp Leafwetness
rep.	16	12	12	3
d.f.	29	29	29	29
l.s.d.	17.22	19.88	19.88	39.76

(Not adjusted for missing values)

Analysis of variance

Variate: Mean_severity_assessment3

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Rep	2	989.312	494.656	61.75	<.001
Temp	3	181.532	60.511	7.55	<.001
Leafwetness	3	130.068	43.356	5.41	0.004
Temp.Leafwetness	9	28.621	3.180	0.40	0.926
Residual	29 (1)	232.320	8.011		
Total	46 (1)	1559.669			

Tables of means

Variate: Mean_severity_assessment3

Grand mean 6.15

Rep	1	2	3
	4.05	12.45	1.94

Temp	5	15	20	25
	5.58	6.61	8.90	3.50

Leafwetness	1	6	24	48
	4.38	4.67	8.15	7.39

Temp	Leafwetness	1	6	24	48
5		3.88	5.00	7.29	6.17
15		3.92	5.38	8.42	8.75
20		8.90	6.10	10.58	10.03
25		0.84	2.23	6.30	4.63

Least significant differences of means (5% level)

Table	Rep	Temp	Leafwetness	Temp Leafwetness
rep.	16	12	12	3
d.f.	29	29	29	29
l.s.d.	2.047	2.363	2.363	4.727

(Not adjusted for missing values)