FINAL
PROJECT REPORT
FOR
HNS 128

To:

Horticultural Development Council
Bradbourne House
East Malling
Kent
ME19 6DZ

Investigation into the epidemiology and control of *Stemphylium* (*Pleospora*) leaf spot of *Hebe*

December 2006

Grower Summary

HNS 128

Investigation into the epidemiology and control of *Stemphylium* (*Pleospora*) leaf spot of *Hebe*.

Project Title:	Investigation into the epidemiology and control of *Stemphylium* (*Pleospora*) leaf spot of *Hebe*.
Project Number:	HNS 128
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Location:	STC Ltd & CSL.
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The results and conclusions in this report are based on a series of laboratory and field trials based at STC Ltd and CSL, York. The conditions under which the experiments were carried out and the results generated have been reported with detail and 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 to be used as the basis for commercial product recommendations.

It should also be noted that many of the fungicide products tested in this work are experimental in nature and may not be currently approved for use on *Hebe* or other HONS species either outdoors or under protection. If anyone is in doubt regarding the current approval status (or crop safety) of a particular product they are considering using they should either, consult the manufacturer, check the status on an approved pesticide database (including the Long Term Arrangements for Extension of Use) or take independent advice from a BASIS qualified adviser.

AUTHENTICATION

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

Signature ……..

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Date ……

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HNS 128 : GROWER SUMMARY

Hebe : Investigation into the epidemiology and control of *Stemphylium* (*Pleospora*) leaf spot.

Headline

This project demonstrated that the large majority of leaf spot infections in the UK are currently caused by the fungus *Stemphylium* rather than *Septoria.* This is the first time this pathogen has been reported on Hebe. Carbendazim e.g. Bavistin used routinely for *Septoria* control was ineffective against *Stemphylium* and fungicide resistance is suspected. Fortunately, several other fungicides were found which effectively controlled the disease and where these have been used in integrated spray programmes effective leaf-spot control has again been achieved.

Background and expected deliverables

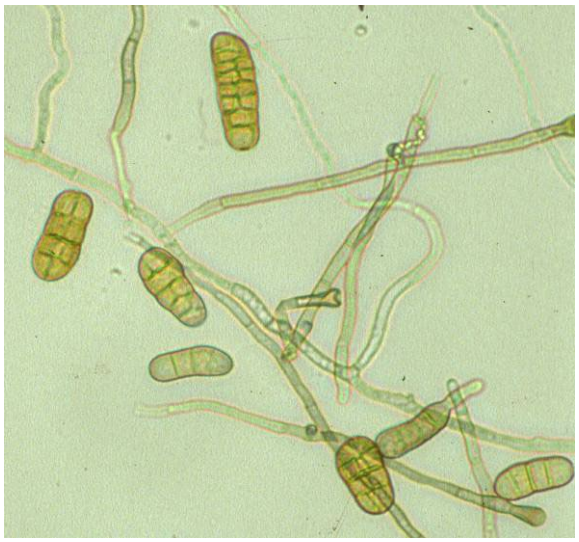
Until recently, leaf spots occurring on *Hebe* cultivars were generally regarded to be due to *Septoria exotica*, and treated with carbendazim (e.g. Bavistin) accordingly. However, on some nurseries, control of *Hebe* leaf spot has been poor following intensive fungicide spray regimes aimed at the control of *Septoria*.

The problem was initially highlighted in spring 2003 by an industry representative who sent samples of badly affected *Hebe* into the Plant Clinic at Stockbridge Technology Centre (Plate 1).

Plate 1. Severe leaf spot infection on *Hebe* cv. Red Edge

Preliminary investigations on samples from the nursery were unable to detect *Septoria* spp. associated with the leaf spots. Instead a different fungus, Stemphylium sp., was isolated (Plate 2).

Plate 2 : Photomicrograph of *Stemphylium* sp. conidia (asexual spores) from isolations carried out at STC in 2003



It quickly became clear that the standard fungicide programme deployed by this particular nursery was having little or no effect in controlling the problem. Broader enquiries during 2003 indicated that leaf spotting on *Hebe* was widespread and that losses on some nurseries were exceptionally high. Without an effective understanding of the disease on hardy ornamental nursery stock species (HONS) it was recognised that plants would continue to be infected and financial losses incurred.

The HDC funded this two year project to investigate different aspects of the disease to provide growers and industry representatives with as much information as possible to reduce the severity of the problem and the financial losses which were being experienced. STC have worked in collaboration with the Central Science Laboratory (CSL) to assist the industry in controlling the problem.

Summary of the Project and Main Conclusions

From information provided by growers it was found that the leaf spot was occurring across the UK and that some varieties were more severely affected than others. It transpired that many growers had assumed the spot to be caused by *Septoria* and had been applying fungicides against this pathogen. The first objective was to identify the pathogen concerned, and carry out fungicide efficacy tests, both in the laboratory, and on outdoor crops. Once the new Hebe pathogen had been confirmed – *Stemphylium sp*. information was circulated about a range of fungicides which were more effective in controlling the pathogen. It was found that iprodione (Rovral), boscalid + pyraclostrobin (Signum), azoxystrobin (Amistar), prochloraz-Mn (Octave) and fluazinam (Shirlan) all significantly reduced the level of infection on Hebe cv 'Red Edge' compared to the untreated control.

As a note of caution: whilst several of the experimental fungicides can be used on HONS subjects via the Long Term Arrangements for Extension of Use, growers are urged to thoroughly check that they are fully complying with statutory conditions of use on the label, e.g. use under protection versus outdoors, prior to application and also to check varietal crop safety by testing on a few plants prior to widespread use on the nursery. Growers should also note that fluazinam (in Shirlan) is a recognised skin sensitiser and perhaps should be avoided where personnel are handling plants.

Other work carried out in the project has investigated:

1. How and under what conditions the disease is spread?
2. Which varieties are most susceptible?
3. The safety of those chemicals that are most effective?
4. The frequency in which treatments should be applied to control the disease?

What has been found out?

1. The disease is mostly spread by air-borne spores (conidia) produced on the leaf spots. Most spores seem to be released during the warm summer months, when temperatures are higher than 25ºC. It is possible that overhead irrigation or rainfall might also assist in spore dispersal.

2. Studies on 10 different cultivars of Hebe investigated whether any varieties were less susceptible to the disease. Although all varieties used in the study could be infected with *Stemphylium* leaf spot, H. vernicosa and Hebe 'Wirimist' did appear to be slightly less susceptible.

3. The four most effective control fungicides were tested on a range of eleven varieties of Hebe at the approved rate and twice the approved rate to see if any of them were likely to cause damage to Hebe crops. Products tested were; Signum, Rovral, Amistar and Octave. The products were applied to outdoor crops. No evidence of crop damage such as scorching, stunting, and twisting, was found following two applications during the summer months[1] even at double the standard rate of application.

[1] Not all cultivars were in flower or came into flower during this study; therefore possible effects on flowers are not known at this time.

4. An investigation was conducted to find out how often fungicides need to be applied to control the leaf spot disease. Signum and Octave were used either alternately or as a tank mix and applied to crops at a range of timings over a season. The timings ranged from fortnightly sprays to a tank mix applied twice during the season. Alternating sprays were found to be as effective as a tank mix of the two products and also that the number of application could be reduced if the timing of applications corresponded with high infection risk periods e.g. periods of higher temperatures. A tank-mix or an alternating strategy ought to minimise the risk of resistant strains developing in the population providing the products selected have contrasting modes of action.

Financial Benefits

Hebe is one of the most important genera from the UK hardy nursery stock growers. While production of large leaved Hebe has always been problematic because of the susceptibility to downy mildew, small leaved Hebe is considered a stable crop in the container grower's product portfolio. Over the past number of years losses from leaf spot have continued to increase. Some growers have ceased production, whilst many have begun to limit the range they produce, dropping the more susceptible varieties. It has been common to see wastage levels well in excess of 20% among susceptible varieties, making production uneconomical. This disease has caused market share reduction and wastage amounting to many hundreds of thousands of pounds per annum. It is hoped that work carried out for this project will have reduced wastage significantly and provided growers with a more effective armoury of fungicides to control Hebe leaf-spot.

Action Points for Growers

- Monitor protected and outdoor stock plants and cuttings, whether bought-in or produced in-house, for early signs of leaf spot symptoms.

- Do not assume all leaf spotting on *Hebe* is necessarily due to *Septoria* as previously reported on this host. Although the grower survey found no incidence of *Septoria* on commercial crops in the period of the study, *Septoria exotica* is still active on established Hebe plants in park & garden settings (McPherson, *pers com*.) so crops also remain at potential risk of this disease.

- Where leaf spot symptoms are found, obtain accurate identification from a commercial diagnostic laboratory to determine if the problem is due to *Stemphylium*, *Septori*a or some other pathogen.

- Ensure effective hygiene around the nursery. Remove older or left over Hebe plants from the nursery, and clear leaf debris from around infected plants as a precaution against carry-over of the disease.

- Where leaf spot is a problem on *Hebe* review your spray schedule and do not rely on the use of carbendazim (e.g. Bavistin) for effective control.

- Consider the situation on your own nursery and, using the information on fungicide efficacy presented consider adjusting your spray schedule but taking note of the statutory conditions of approval on the label and the Long Term Arrangements for Extension of Use as they apply to non-edible (ornamental) crops.

- The evaluation of varietal crop safety with the novel fungicides that has been carried out on a range of commercially available Hebe varieties did not indicate any obvious crop safety risks. However, growers should exercise caution when applying new fungicide (and other) products to new varieties and crops by test treating a few plants, preferably a range of varieties, in the first instance.

- Growers should monitor their Hebe crops for leaf spot to be aware of the potential level of inoculum present. Application of crop safety products to control leaf spot should be timed to follow high infection risk periods (max daily temps at or above 25ºC).

SCIENCE SECTION

Introduction

Leaf spotting in *Hebe* cultivars is not a new phenomenon, although historically the spotting in *Hebe* has been caused by the pycnidial fungal pathogen *Septoria exotica*.

Plate 3. Characteristic leaf spot symptoms of *Septoria exotica* lesions on *Hebe*

During the spring and summer of 2003 samples of *Hebe* exhibiting a severe leaf spot were received from a regular client of the STC Plant Clinic. On each occasion *Stemphylium* sp. (initially thought to be *Stemphylium botryosum*) was diagnosed as the predominant fungus. *Stemphylium* spp., especially *S. botryosum* are more frequently seen as saprophytic or secondary opportunistic fungi rather than true pathogens of *Hebe* and initially the diagnosis was therefore questioned, because the fungus might have been colonising the leaf tissues secondarily following invasion by other documented primary pathogens such as *Septoria*. The grower also reported having little success in controlling the leaf spot with his standard regime of fungicides. During the late summer the same grower informed us that over a weekend period of high humidity the vast majority of *Hebe* cv. 'Red Edge' had all become severely infected with the *Stemphylium* leaf spot. The grower kindly agreed to donate the plants to STC for further study and investigation.

Plate 4 : Characteristic symptoms of *Stemphylium* leaf spot on *Hebe* cv. 'Red Edge'

Plate 5 : Characteristic growth of *Stemphylium* sp. isolated from *Hebe* on agar

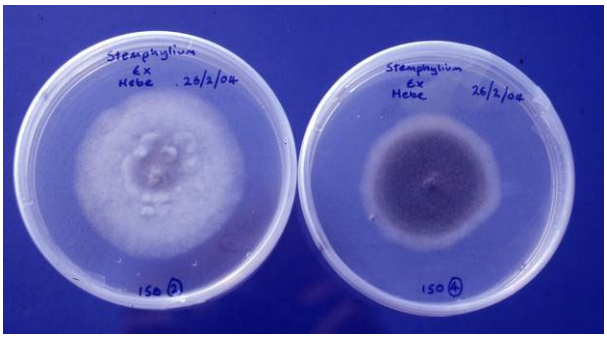


Plate 6 : Characteristic spores of *Stemphylium* sp. isolated from *Hebe* on agar

Preliminary fungicide resistance testing in the Plant Clinic at STC used 5 isolates of *Stemphylium sp*. collected from the infected stock of *Hebe* cv. 'Red Edge' to gain a clearer understanding of the problem. These laboratory-based tests enabled us to provide the grower with some suggestions for alternative chemicals to aid control of the problem. However, as the investigation progressed, it became clear that we may be dealing with a potential new pathogen on *Hebe* and that further in-depth investigation was required. A collaborative HDC-funded project between STC and CSL was therefore developed to investigate the true identify of the causal organism, to study pathogen epidemiology and fungicide sensitivity so that a range of cultural and chemical control measures might be recommended to the industry.

Materials & Methods

Preliminary Grower Survey

An information sheet outlining the symptoms of the new leaf spot in *Hebe* and its possible causes was produced and sent to 25 commercial *Hebe* growers (names and addresses kindly supplied by Mr D Elliott) along with a detailed questionnaire and a request for any samples of *Hebe* material with leaf spot symptoms (Appendix 5). A response rate of 72% suggests that many growers were experiencing significant problems in *Hebe* production and seeking answers. Microscopic examination and fungal isolations onto a range of artificial media were carried out by STC and CSL staff. Cultures isolated from plants with leaf-spot symptoms were retained in a collection held at CSL. The information gleaned from this initial survey provided the impetus for a longer-term project to try and elucidate the nature of this new problem in the HONS industry.

Objective a : Isolation and identification of the pathogen

Independent isolations by STC and CSL consistently yielded a *Stemphylium* sp. from the samples submitted during the grower survey. This supported the observations of the preliminary investigation at STC. To try and identify the species involved CSL took four representative cultures of the *Stemphylium* sp. and extracted the total DNA. ITS regions 1 and 2 of the rRNA gene were then purified and sequenced. Sequence data was subsequently compared with the NCBI (National Centre for Biotechnology Information) database of sequences to try and secure a match with known species.

Objective b : Demonstrating Koch's postulates

Stemphylium spores produced from Tap Water Agar cultures from isolations from Hebe cv Red Edge were harvested and diluted to a final concentration of 1×10^5 - 1×10^6 spores/ml in sterile distilled water (SDW). A 10µl drop of spore suspension was placed on the wounded and unwounded upper leaf surface of young containerised *Hebe* cv. Red Edge plants at CSL. This was repeated for 10 leaves/plant. Drops of SDW without spore suspension were used as controls on separate plants. Test plants were incubated at 18°C, 16hr light/8hr dark and high (>95%) humidity and monitored daily for symptoms. Where symptoms were found they were recorded in detail and re-isolations of the *Stemphylium* sp. attempted in support of Koch's postulates.

Objective c : Examining spore dispersal

A series of studies to investigate spore dispersal mechanisms in *Stemphylium* was undertaken during 2004-2005. Initial experiments used an exposed agar plate method of spore trapping and

focused on spore release at different times of day to determine if there was any diurnal rhythm to spore release. Plates (5 replicates of ¼ strength Potato Dextrose Agar amended with Lactic Acid) were positioned above crop of Hebe plants in a polytunnel at 5 different times during the day. The plates were left exposed for 20 minutes then incubated for 2-4 days prior to colony examination. This test was repeated on 3 occasions during July 2004 and again on 6 occasions in 2005.

The same experiments were subsequently repeated on several occasions during November 2004, where the influence of irrigation/rainfall was also superimposed on the time of day. Two trays of infected plants were used during this experiment. One tray was irrigated using a medium rose attachment on a hose, whilst the other tray was not watered during the experimental period. Following irrigation, agar plates were positioned on the top of both trays of plants to trap any spores released during 4 x 20-minute periods at 11.00, 11.05, 11.25 and 11.45.

Objective d : Studying infection conditions for the *Stemphylium* sp.

Three *Stemphylium* isolates (Ref No.'s 2212, 2216 and 2220) were tested *in vitro* for their sensitivity to temperature. The growth of each isolate was examined on tap water and potato dextrose agars at 6, 8, 11, 16, 20, 24 and 30°C. For each isolate, temperature regime and agar type three replicate plates were inoculated at the centre of the plate with a 5mm agar plug taken from the leading edge of 10 day old *Stemphylium* colony. Plates were incubated in the dark and measurements of colony diameter taken after 1, 2, 7 and 13 days.

Objective e : Investigating the role of leaf litter in disease epidemiology

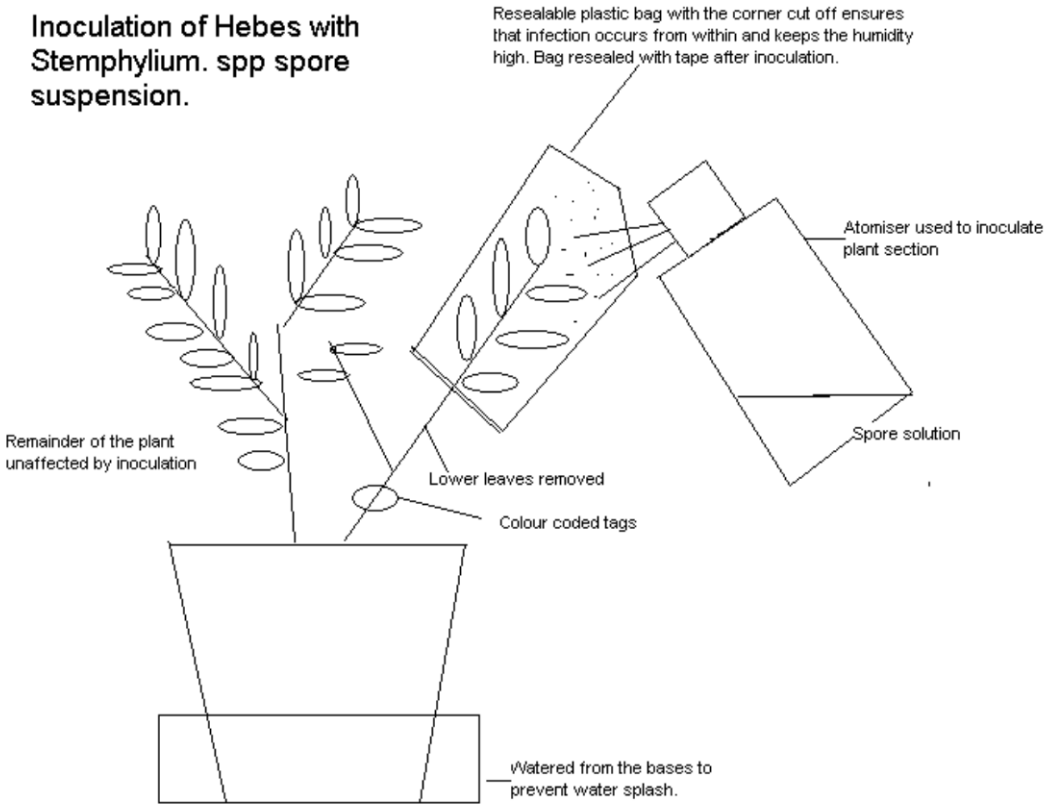
Leaf litter samples were collected from commercial nurseries at different times during the year and initially examined for the presence of the sexual stage of the pathogen. The samples were then incubated under high RH conditions to induce further development and re-examined at intervals using microscopy (x 100 & x400).

Objective f : Studying varietal susceptibility to the disease

Differences in varietal susceptibility to *Stemphylium* sp. was tested against ten Hebe varieties and types (H. 'Wirimist', H. vernicosa, H. rakaiensis, H. 'Red Edge', H. 'Sweet Kim', H. albicans, H. pageii, H. 'Baby Marie', H. 'Champion', H. 'Silver Dollar'). For each Hebe variety examined, re-sealable plastic bags were placed over six stems/plant, the corner of each bag removed and four branches inoculated with 3 ml of a conidial suspension containing $1x10^4$ spores per ml of *Stemphylium* isolate 2208, 2165, 2211 or 2217. The fifth branch was inoculated with a mixed

conidial suspension and the sixth branch with water as a control (Figure 1). The bags were sealed to maintain a high relative humidity and the plants incubated in a controlled environment room set at 18°C (Plate 7). Symptom development was measured after 20 days using a categorical scale where 0 = no symptoms, 1 = slight, 2 = moderate, and 3 = severe.

Figure 1. Schematic of the methodology for testing varietal susceptibility. Plate 7 shows a plant inoculated by this methodology, and incubating prior to assessment.



NB. The schematic diagram shows the methodology used, however in practice more than 1 shoot/plant was inoculated in this way.

Plate 7. Hebe plant incubating following inoculation by the method outlined in Figure 1

Objective g : *In vitro* fungicide screening

An initial *in vitro* screen of fungicides was carried out at STC using 5 isolates of *Stemphylium* from the same site. These tests were subsequently repeated using 5 isolates of *Stemphylium* collected from infected *Hebe* plants all from different sites. The new isolates were chosen to represent crops in different parts of the UK to determine if there was any geographical variability in the genotype of the *Stemphylium* sp., which might make it more or less susceptible to fungicides. Table 1 shows details of the isolates used in the *in vitro* fungicide screen.

Table 1. *Stemphylium* isolates used during the in vitro fungicide screen

Isolate Number	STC Clinic No.	*Hebe* cultivar/type	Geographical location
1	PC 3323C	H. 'Silver Dollar'	Wales
2	PC 3348b	H. pageii	Yorkshire
3	PC 3350c	H. 'Silver Dollar'	Leicestershire
4	PC 3372	H. rakaiensis	Middlesbrough
5	PC 3373c	H. 'Caledonia'	Worcestershire

All *in vitro* fungicide resistance screening was carried out using agar amended with fungicides at 0, 2, 20 and 100ppm (parts per million) concentrations. This test compares the inhibition of radial growth of mycelium between the fungicide amended agar (PDA) plates and the unamended plates (0ppm). Plugs (5mm diameter) from 3-4 day old cultures of *Stemphylium* were placed centrally on the agar (3 replicates/concentration/isolate) and incubated at ambient temperature. Measurements of the radial growth of the fungus were made after 3 days.

Table 2. Fungicides evaluated in the *in vitro* laboratory screen

Active Ingredient	Chemical Group	Product	Manufacturer
azoxystrobin	strobilurin (QoI)	Amistar	Syngenta
boscalid + pyraclostrobin	anilide+strobilurin (QoI)	Signum	BASF
carbendazim	benzimidazole (mbc)	Bavistin	BASF
chlorothalonil	Chlorophenyl	Bravo	Syngenta
iprodione	dicarboximide	Rovral	BASF
fluazinam	Pyridinamine	Shirlan	Syngenta
mancozeb	dithiocarbamate	Karamate	Landseer
mepanipyrim	anilinopyrimidine	Frupica	Certis
myclobutanil	Triazole	Systhane	Landseer
prochloraz	Triazole	Octave	Scotts
pyrimethanil	anilinopyrimidine	Scala	BASF
tebuconazole	Triazole	Folicur	Bayer Crop Science
tolylfluanid	Multi-site	Elvaron Multi	Bayer Crop Science

N.B. For more information on fungicide groups refer to the FRAG-UK web-site(http://www.pesticides.gov.uk/rags.asp?id=644)

Objective h : *In vivo* fungicide comparisons

A batch of *Hebe* cv. 'Red Edge' with a moderate-severe infection with *Stemphylium* leaf spot was arranged in a randomised plot layout consisting of 12 treatments, each with 4 replicates. Each treatment (4 replicate plots) was comprised of 2 plots containing 20 small *Hebe* plants (1 litre pots) and 2 plots containing 15 large *Hebe* plants (2 litre pots) with a total of 35 plants/plot. Fifteen shoots were randomly selected from each plot and, at the commencement of the trial, the division between *Stemphylium*-infected and healthy growth was marked by positioning an elastic band around the stem (Figure 2).

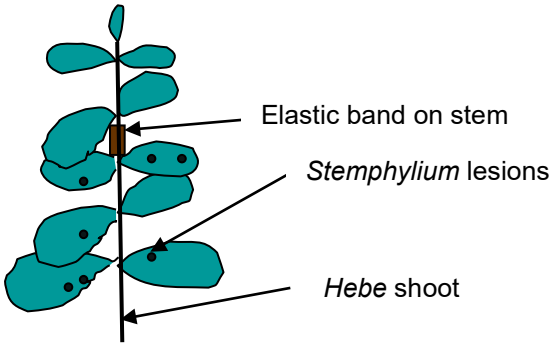


Figure 2 : Schematic diagram of a *Hebe* shoot with an elastic band affixed between infected and uninfected tissue.

Treatments

Four fungicide applications were made at 14-day intervals using a Hozelock Premier 5 litre sprayer.

The spray applications were applied to the point just before run-off.

Table 3: Details of the fungicide treatments used in the field trial

Treatment	Active Ingredient	Rate of application	Water rate (l/ha)
1. Water control	-	-	250
2. Bavistin	carbendazim	1.0 g/l	250
3. Rovral WP	Iprodione	1.0 g/l	250
4. Signum	boscalid + pyraclostrobin	1.8 kg/ha	250
5. Shirlan*	fluazinam*	1.5 l/ha	250
6. Scala	pyrimethanil	0.1 g/l	250
7. Octave	Prochloraz	2.0 g/l	250
8. Folicur	tebuconazole	1.0 l/ha	250
9. Plover	difenoconazole	1.0 l/ha	250
10. Systhane 20EW	myclobutanil	0.3 ml/l	250
11. Amistar	azoxystrobin	1.0 l/ha	250
12. Frupica	mepanipyrim	1.0 kg/ha	250

** reports of skin sensitization with some workers handling treated plants in some crops.*

Crop Diary

13.09.04	Replicated *Hebe* trial laid-out
14.09.04	Baseline disease assessment conducted
16.09.04	1st Fungicide application
05.10.04	2nd Fungicide application
18.10.04	Interim disease assessment conducted
18.10.04	3rd Fungicide application
04.11.04	4th Fungicide application
15.11.04	Interim disease assessment conducted
04.02.05	Final disease assessment conducted

Assessments

An initial disease assessment was carried out to determine the number of leaf pairs from the apex to the first pair of infected leaves, and the number of lesions on the first pair of infected leaves on the marked shoots. Subsequent assessments recorded the number of pairs of healthy leaves, disease lesions and the total number of leaf pairs above the band (a measure of the growth) and the number of disease lesions on the side shoots (Figure 3).

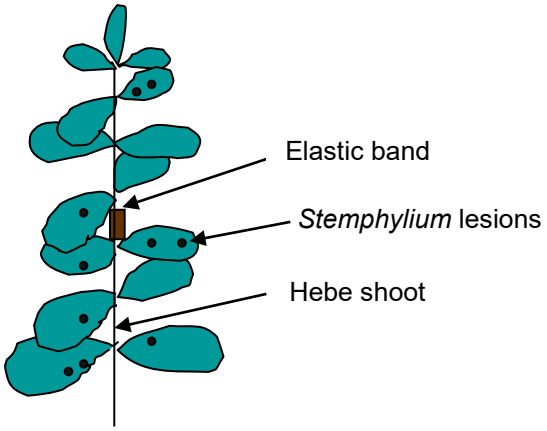


Figure 3: Schematic diagram of a *Stemphylium*-infected *Hebe* shoot. The shoot score: total number of leaf pairs above band = 4; number of healthy leaf pairs from the apex = 1; and, the number of disease lesions = 3.

Statistical Analysis

Data from the replicated trial was analysed using ARM trial management software and Excel (Microsoft Office 2003).

Objective I : Ensuring crop safety with applied fungicides

An unreplicated trial which comprised of 9 treatments, including 4 fungicides applied at 1N & 2N rates of application together with an untreated control was carried out at STC during the early autumn 2005.

A range of 11 cultivars were chosen for their susceptibility to leaf spot, their potential vulnerability to chemicals (soft foliage) and their current or anticipated popularity in the market.

Cultivars chosen were:

H. rakaiensis
H. vernicosa
H. 'Wirimist'
H. 'Baby Marie'
H. 'Mrs Winder'
H. pageii
H. albicans
H. 'Red Edge'
H. 'Sweet Kim'
H. macrocarpa latisepala
H. 'Pink Paradise'

Each plot contained two plants of each cultivar (24 plants in total).

Table 4. Fungicide treatments applied during crop safety trial – 2005

Product	Active Ingredient	1N rate	2N rate	Water rate (l/ha)
Untreated	-	-	-	
Signum	pyraclostrobin + boscalid	1.8 kg/ha	3.6 kg/ha	250
Rovral	iprodione	1.0 g/l	2.0 g/l	250
Amistar	azoxystrobin	1 l/ha	2 l/ha	250
Octave	prochloraz	2 g/l	4 g/l	250

A total of 2 applications were carried out on the 2nd & 16th September.

A visual assessment of the crop prior to each application and 2 weeks following the last application was carried out to check for any symptoms that may have been caused in response to the treatment applications.

Objective j : Investigating fungicide timing relative to the infection cycle

An investigation was carried out in the period July – December 2005 to evaluate the efficacy of a range of application timings of proven crop protection products to determine if effective disease control could be maintained with reduced fungicide inputs.

The trial comprised of 13 treatments. Each treatment contained 4 replicate plots fully randomised in a blocked design. Each plot contained 14 plants of the Hebe cultivar 'Red Edge'.

Table 5. Fungicide Treatments applied during timing trial – 2005

Treatment No.	August		September		October		November	
	1st App	2nd App	1st App	2nd App	1st App	2nd App	1st App	2nd App
1	-	-	-	-	-	-	-	-
2	☑	☑	☑	☑	☑	☑	☑	☑
3	☑	-	☑	-	☑	-	☑	-
4	☑	-	-	☑	-	-	☑	-
5	☑	-	-	-	-	-	-	-
6	☑	☑	-	-	-	-	-	-
7	✓S	✓O	-	-	-	-	-	-
8	☑	☑	-	-	☑	☑	-	-
9	✓S	✓O	-	-	✓S	✓O	-	-
10	-	-	☑	☑	-	-	☑	☑
11	-	-	✓S	✓O	-	-	✓S	✓O
12	-	-	☑	☑	-	-	-	-
13	-	-	✓S	✓O	-	-	-	-

✓ = Alternating programme of full rate Signum or Octave ☑ = Tank Mix of Signum + Octave at ½ rate

All treatments comprised of either a Signum and Octave tank mix at 50% rate or an alternating programme of the same products applied at the full label rate. Applications were carried out using a Hozelock Premier 5L sprayer.

An initial disease assessment was carried out to determine the number of leaf pairs from the apex to the first pair of infected leaves, and the number of lesions on the first pair of infected leaves on the marked shoots. Subsequent assessments recorded the number of pairs of healthy leaves, disease lesions and the total number of leaf pairs above the band (a measure of the growth) (Figure 4).

Crop Diary

04.08.05 Pre-spray disease assessment carried out and plants tagged.
05.08.05 1st spray application carried out.
19.08.05 2nd spray application carried out.
02.09.05 3rd spray application carried out.
16.09.05 4th spray application carried out.
27.09.05 Disease assessment conducted
03.10.05 5th spray application carried out.
17.10.05 6th spray application carried out.
31.10.05 7th spray application carried out.
11.10.05 8th and final spray application carried out.
07.12.05 Disease assessment conducted

Statistical Analysis

Data from the replicated trial was analysed using ARM trial management software.

Objective k : Preparation of an Industry fact sheet on leaf diseases of *Hebe*.

A Fact sheet, providing information on the various leaf diseases of *Hebe*, including *Stemphylium* leaf spot, has been prepared and will be available to growers in the near future.

Results

Preliminary grower survey

A large number of records describing cultivar range, spot severity relative to cultivars, fungicide regimes, irrigation methods, and history of leaf spot outbreaks on nurseries was gathered from the survey responses; details are provided in Appendix 1. The symptoms on most of the grower samples submitted were relatively similar and conformed closely with those seen earlier (Plates 8a-f) and most samples yielded a *Stemphylium* sp.. The leaf spots were discrete, brown-black in colour and with a central ash coloured centre on older lesions. None of the samples examined were infected with *Septoria* leaf spot.

Plates 8a-f : A range of leaf spot symptoms on samples submitted in the initial grower survey.

(a)

(b)

(c)

(d)

(e)

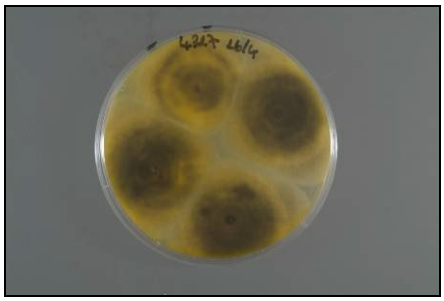
(f)

Objective a : Isolation and identification of the pathogen

Isolations carried out in tandem by STC and CSL on the submitted samples resulted in a total of 39 positive *Stemphylium* sp. identifications from 50 samples (78% positive for a *Stemphylium* sp.). The presumptive diagnoses in this study were based on either the production of conidia characteristic of this fungus (Plate 2), or based on colony morphology of *Stemphylium* in conjunction with a characteristic yellow staining of the agar in culture (Plates 9a-b).

Plates 9a-b : Characteristic colony morphology of the *Stemphylium* sp. isolated from *Hebe* leaf spot

(a) top view of colonies



(b) view of colonies from below

A selection of other organisms such as *Botrytis, Cladosporium* and bacterial species were detected in a few samples, though these were not regarded to be of particular pathological significance in relation to the leaf-spot symptoms. Importantly, no evidence of *Septoria exotica* was detected on any of the samples submitted.

Using modern molecular techniques at CSL a 96% match to *Stemphylium solani* was made during comparisons with national database.

Objective b : Demonstrating Koch's postulates

Following artificial inoculation as described, necrotic spots were observed principally at the edge of the inoculation drop after 2 days incubation (Plate 10).

Plate 10 : Initial symptoms of leaf spot infection following artificial inoculation with *Stemphylium* sp.

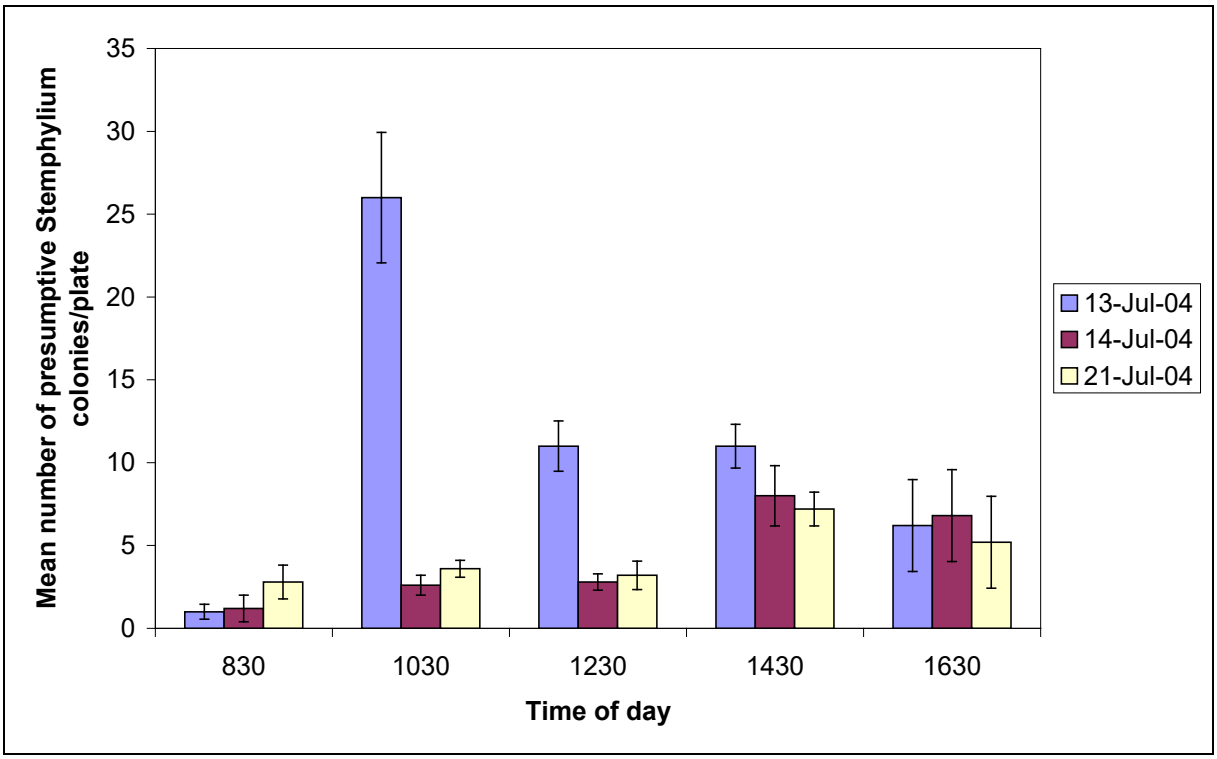
Lesions expanded and developed over the next 7 days on all inoculated leaves (see Plate 11). Lesion development was seen both with and without wounding and this suggests that the pathogen is able to enter the host tissue either via natural openings (stomata) or via the production of an infection peg. Necrosis was not observed on the uninoculated control leaves. Preliminary observations, prior to formal experiments to demonstrate Koch's postulates, indicated that lesion development was significantly reduced when the relative humidity was lower. A *Stemphylium* sp., conforming to that introduced in the inoculation experiments was successfully re-isolated from the necrotic leaf tissues, thereby satisfying Koch's postulates.

Plate 11 : Established leaf spot lesions on *Hebe* cv. 'Red Edge' following artificial inoculation with *Stemphylium* sp. 9 days earlier.

<u>Objective c : Examining spore dispersal</u>

Following exposure in an infected crop area, the agar plates were monitored for 2-4 days to check for growth of *Stemphylium*-like colonies. In most cases, a presumptive diagnosis based on the yellow staining of the agar (previously observed with *Stemphylium* cultures), was used, because the colonies were quickly overgrown by more aggressive or more prevalent organisms. The results for the initial spore trapping are presented in Figure 4.

Figure 4. Incidence of *Stemphylium* spores over an 8hr period on 3 dates

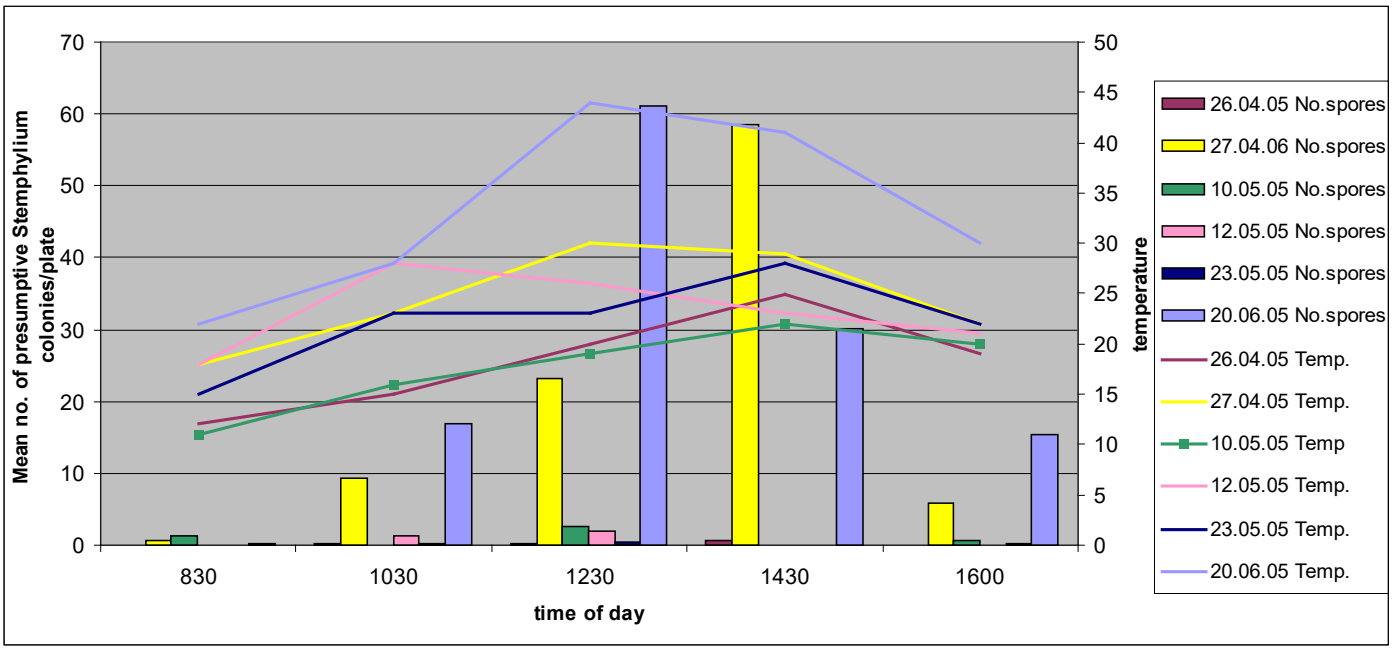


Statistical analyses of these data indicate that there was no significant difference in the number of spores released in the crop throughout the day. However, the plotted data (Figure 4) tends to suggest that, with one exception, there were more spores released in the afternoon than during the morning and this perhaps signifies a temperature response for spore release.

During November and early December 2004 the spore trapping experiments were repeated with plates being exposed in the crop for 20-minute periods followed by either a period of overhead irrigation or no irrigation. Despite the high level of repetitions of the test, virtually no spores were trapped during the November to early December period. This would suggest that the potential for severe leaf-spot infection during the winter months is relatively low.

Further spore trapping experiments were repeated on 6 occasions during spring and early summer 2005 (Figure 5). The data generated suggests that the greatest spore release occurred on days when the temperature was higher (27.4 & 20.5) and on each day it peaked around midday (1230-1430hrs).

Figure 5. Monitoring Spore release of *Stemphylium* sp. during Spring 2005



Objective d : Studying infection conditions for the *Stemphylium* sp.

Growth of the three isolates evaluated in this study was sensitive to temperature with least colony growth measured at 6°C and most at 30°C (Figure 6). Data were combined across the temperature range (Figure 7). A logistic curve could be used to describe the relationship between accumulated temperature and colony growth. Comparison, by ANOVA, of the parameters for these individual curves showed that a single line was adequate to describe the response of all three isolates (R^2=94.5%). This curve has the form:

$$C = -46.7 + \frac{133.7}{1 + \exp(-0.00853(T - 87.1))}$$

Where C=colony diameter
T=accumulated temperature

Figure 6. Colony growth of three *Stemphylium* sp. isolates on PDA at different temperatures.

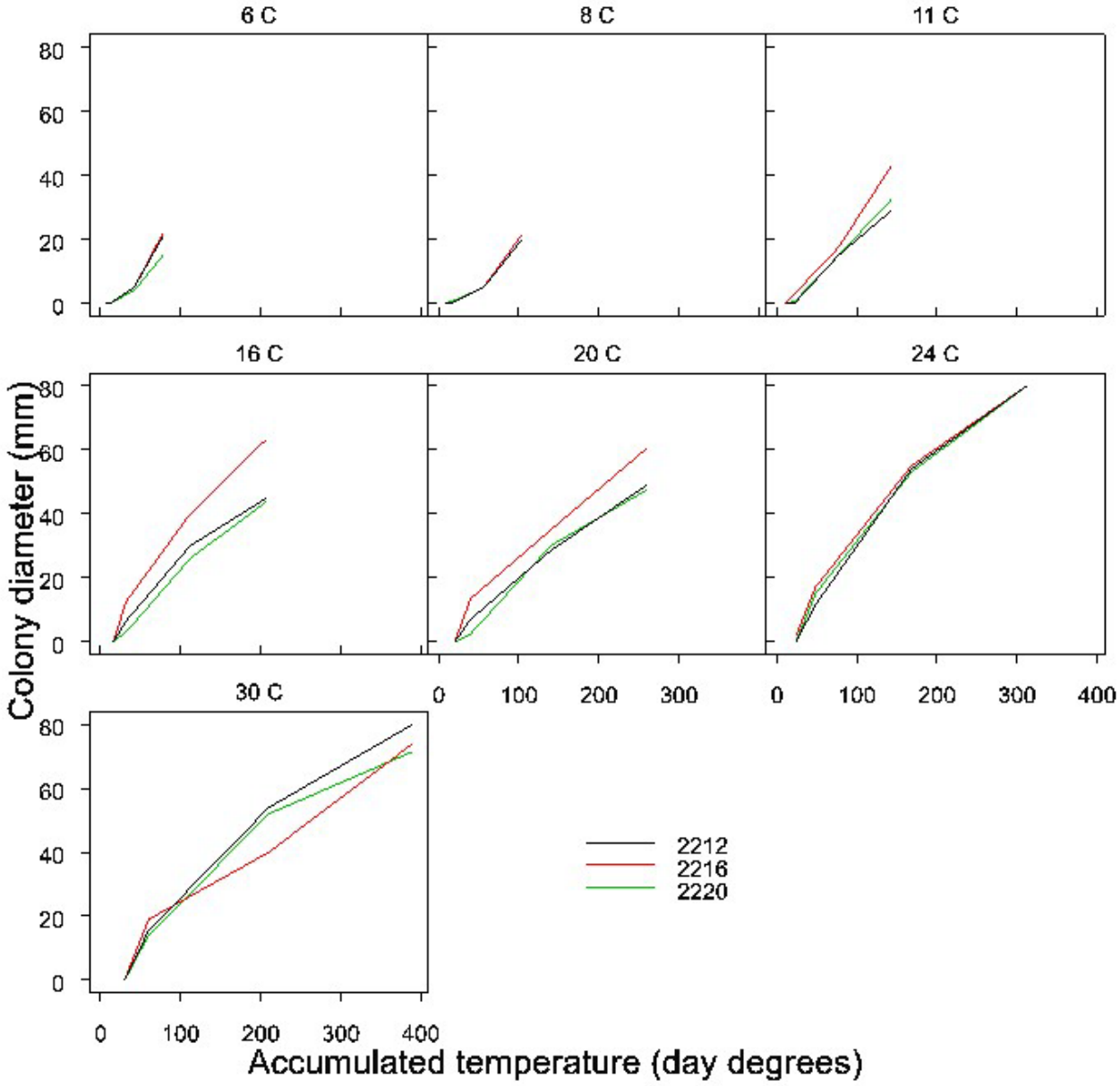
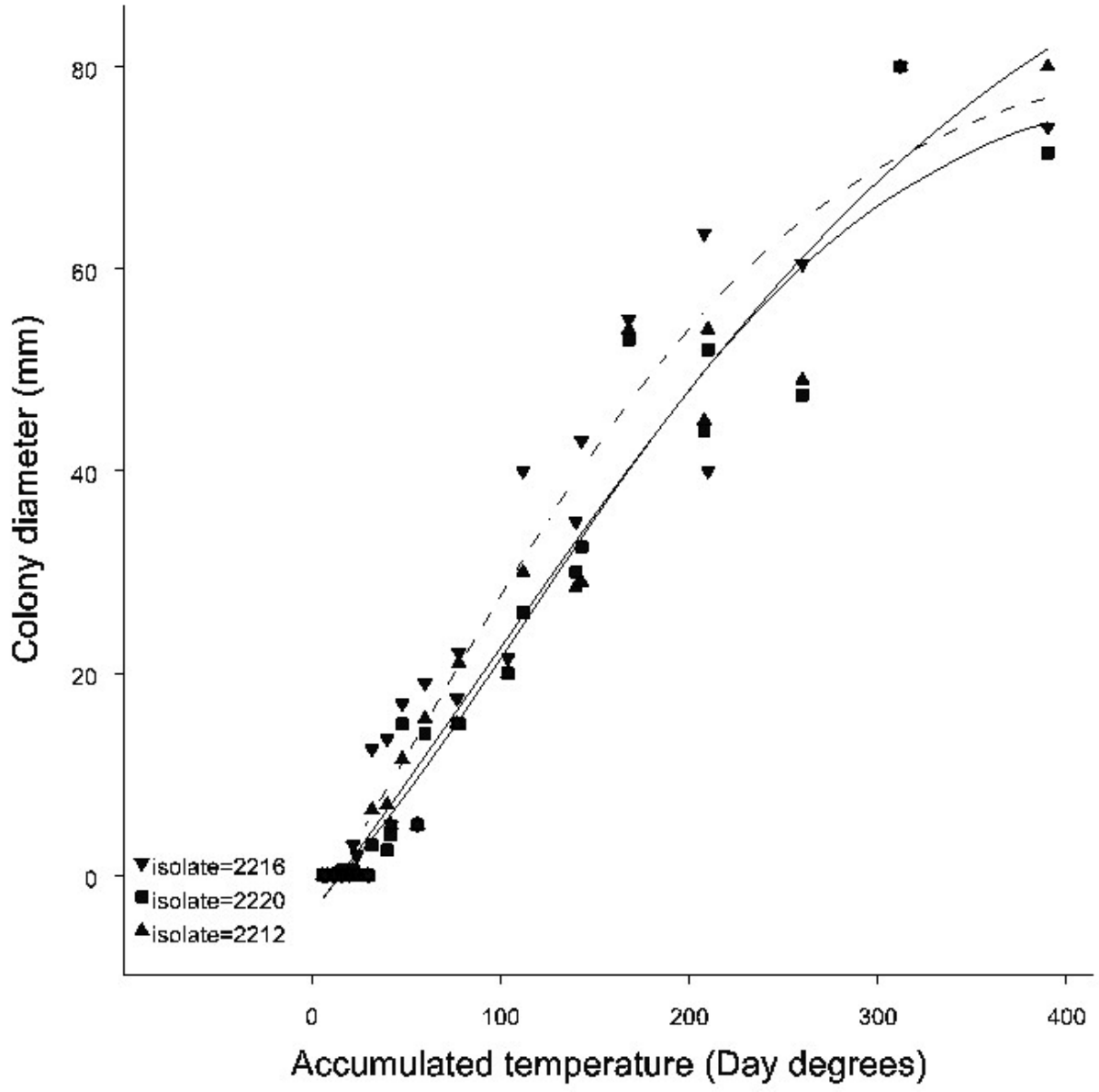


Figure 7. Response of three isolates to temperature. Lines show the logistic curve fitted for each isolate. No statistical difference was found between these lines, so a single line can be used to describe the response to temperature across the three isolates (R^2=94.5%, see text).



Objective e : Investigating the role of leaf litter in disease epidemiology

Samples of leaf litter collected from infected sites have been monitored regularly by retaining infected leaves under contrasting wet and dry conditions. During this period the sexual or perfect stage of the fungus (*Pleospora*) was not detected on any of the 6 samples collected at various times of year.

Objective f : Studying Varietal Susceptibility to the Disease

None of the cultivars tested were immune to *Stemphylium* though some differences in susceptibility to the disease following artificial inoculation were noted (Table 6). For example, Plate 12 shows typical symptom development on the cultivar *H. albicans*. However, relative to this, leaf-spot symptoms on the cultivars *H. vernicosa* and *H.*' Wirimist' were relatively minor (Table 6). See Appendix 6 for detailed photographic records.

Table 6. Varietal susceptibility[+] to three isolates of *Stemphylium.*

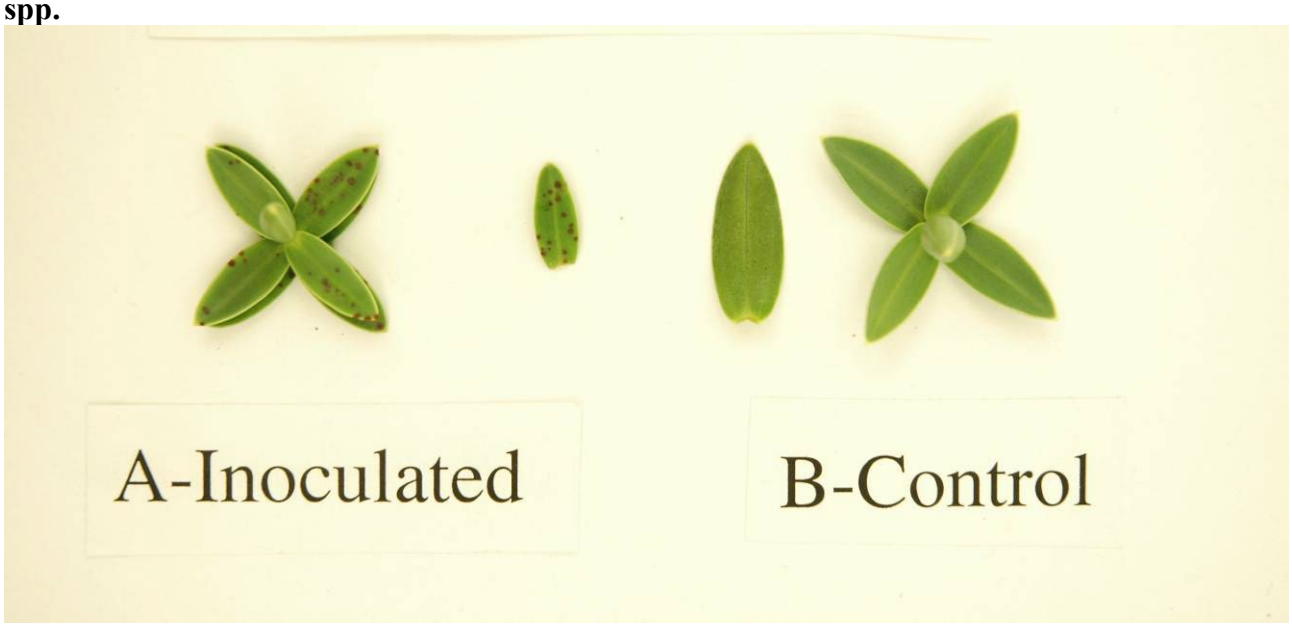
Variety		Isolate[1]				
	Control	2208	2165	2211	2217	mixed
H. albicans	0	1	2	3	3	3
H. 'Baby Marie'	0	3	3	3	3	3
H. 'Champion'	0	1	1	1	2	2
H. 'Garden Beauty Blue'*	-	-	-	-	-	-
H. 'Gold Beauty'*	-	-	-	-	-	-
H. macrocarpa*	-	-	-	-	-	-
H. 'Mrs Winder'#	-	-	-	-	-	-
H. pageii	0	0	1	1	1	1
H. rakaiensis	0	2	2	2	2	2
H. 'Red Edge'	0	1	1	2	3	3
H. 'Silver Dollar'	0	1	1	3	3	3
H. 'Sweet Kim'	0	1	1	1	2	2
H. vernicosa	0	1	1	0	1	1
H. 'Wirimist'	0	1	1	1	0	1

[+] Susceptibility measured on a 4 point (0-3) categorical t scale where 0 = healthy, 1 = slight, 2 = moderate, 3 = severe

* Not screened – samples unsuitable due to poor plant quality (high levels of *Stemphylium* leaf spot).

- Persistent aphid infestation, assessment not possible

Plate 12. Comparison of variety *H. albicans* inoculated with mixed isolates of *Stemphylium* spp.



Objective g : *In vitro* fungicide screening

The *in vitro* fungicide screen gave very clear results regarding chemical sensitivity (Figure 8). Perhaps of most significance is the fact that all isolates of *Stemphylium* tested were totally insensitive to carbendazim (Bavistin), signifying either a high level of resistance in the pathogen population (or alternatively an inherent insensitivity to the active ingredient). Baseline studies using wild type isolates not previously exposed to the fungicide would be required to determine this. In contrast, several of the alternative fungicides tested provided a high level of inhibition of mycelial growth at the lowest concentration (2ppm) of active ingredient. Those most effective in this *in vitro* laboratory study were iprodione (Rovral), boscalid + pyraclostrobin (Signum), fluazinam (Shirlan) and pyrimethanil (Scala) and all resulted in >70% inhibition of mycelial growth at 2ppm. The results for the protectant fungicide chlorothalonil (e.g. Bravo 500), which is often used as a component of nursery spray regimes, performed poorly with approximately 10% inhibition of growth at 100ppm active ingredient (See Appendix 2 for full data sets). Fungicides which showed moderate inhibition included prochloraz-Mn (Octave), myclobutanil (Systhane) and tebuconazole (Folicur).

Data from this set of experiments closely mirrored the results for 5 random isolates of *Stemphylium* collected from one nursery in 2003 and used in preliminary studies by STC.

A comparison of the relative sensitivity of the different geographic isolates to the range of fungicides tested was made though no clear patterns emerged. This suggests that geographical differentiation is not occurring at present. However, it may be interesting to re-visit this work in

future years to monitor any shifts in relative sensitivity of the pathogen relative to the fungicide spray programmes deployed on different nurseries.

Objective h : *In vivo* fungicide comparisons

The baseline leaf-spot assessment carried out in the experimental crop prior to the commencement of fungicide applications indicated moderate infection levels with little variation seen either in the mean number of lesion-free leaf pairs/plant (i.e. on the leaf pairs above the marker band) or in the mean number of lesions recorded on the uppermost pair of leaves on each plant (Full data in Appendix 3, Table 1a). A disease assessment carried out at the mid-point of the experiment following two fungicide applications (Appendix 3, Table 2a) indicated a significant reduction (P=0.05) in *Stemphylium* leaf-spot following applications of Rovral (iprodione), Signum (boscalid + pyraclostrobin), Shirlan (fluazinam), Octave (prochloraz) and Amistar, when compared to the untreated (water) control (Treatment 1).

A further assessment was conducted 11 days after the final (4^{th}) fungicide application. The data from this assessment (Appendix 3, Table 3a) indicated that the growth of the plants in each treatment was broadly similar and this, together with a lack of any visible effect, suggests that the applied treatments were not phytotoxic. It also indicates that the leaf-spot pathogen was not having any detrimental impact on the rate of plant shoot growth, but instead simply reduces plant quality in the absence of effective fungicides (see also Objective l)

The spread of *Stemphylium* leaf-spot up the *Hebe* shoots was significantly reduced by several of the fungicide applications. Signum, Rovral, Shirlan, Octave and Amistar were the most effective fungicides against *Stemphylium* throughout the study. The suppression of the disease with these fungicides was significantly better (P=0.05) than all other treatments. The fungicides Bavistin, Scala, Folicur and Systhane proved largely ineffective in controlling the development of *Hebe* leaf spot, in the experiment. The results for both pyrimethanil and tebuconazole were disappointing as the *in vitro* studies indicated effective inhibition of mycelial growth.

Signum (pyraclostrobin + boscalid) was found to provide good protective action against *Stemphylium* infection on newly developing shoots. The mean number of healthy leaves on Signum treated plants was significantly improved compared to the control, Bavistin, Rovral, Scala, Folicur, Plover and Systhane treatments.

The results obtained in this study therefore indicate there are a number of potential fungicides that could be used instead of carbendazim to provide effective control against *Hebe* leaf spot caused by *Stemphylium* sp.

Ultimately, a disease assessment in early February 2005, 3 months after the final fungicide application (Appendix 3 table 4a) suggests that Signum maintained a good long-term suppressive action over the *Stemphylium* leaf spot. Significantly lower levels of infection were observed in Signum treated plants, compared with both the untreated control plants and several of the other fungicide treatments including Bavistin, Scala, Folicur and Systhane. Shirlan and Octave were only marginally less effective than Signum, whilst Amistar and Rovral also showed moderate disease suppression at this time. Plants treated with either Signum or Shirlan both resulted in significantly more healthy leaf pairs/plant than many of the plants treated with alternative fungicides. No differences were observed in the total number of leaf pairs produced across the treatments.

Whilst both the efficacy and crop safety studies with the selected fungicides have been very encouraging, growers are urged to proceed with fungicide application with a degree of caution and preferably test treat a few plants of each cultivar they are growing in the first instance as different conditions on a range of different cultivars could produce different effects. Also, growers are urged to be aware of the potential risk to personnel handling plants following application of fluazinam based products due to previous reports of skin sensitisation in particular individuals. Finally, whilst several of the fungicides can be legally applied to HONS via the Long Term Arrangements for Extension of Use growers must make sure they are fully conversant, and compliant, with the statutory conditions of use on the label in all cases prior to application.

Figure 8. ***In vitro*** **fungicide screen – percentage inhibition of mycelial radial growth on agar in the laboratory**

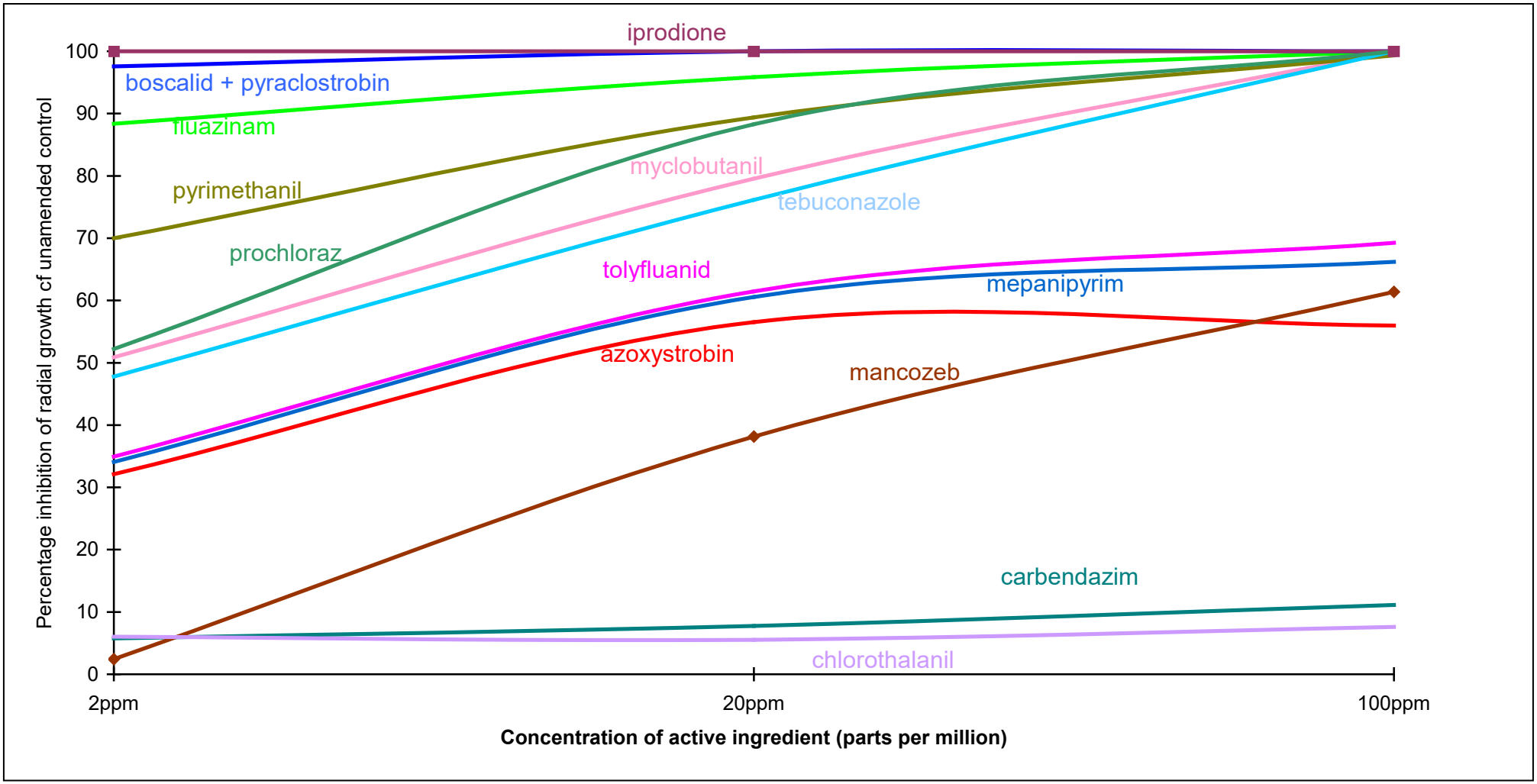
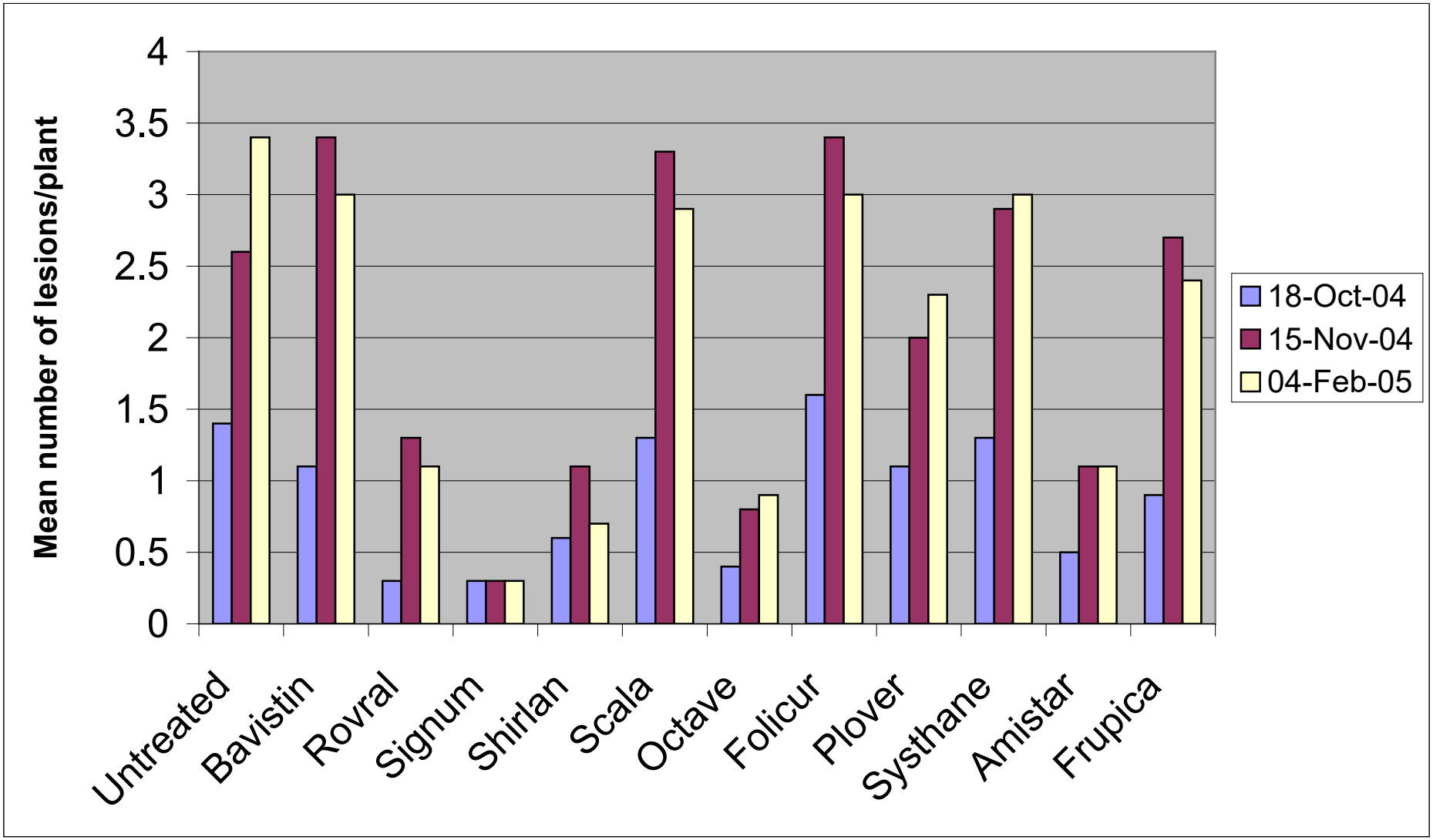


Figure 9 –Comparison of Fungicides for the control of *Stemphylium* leaf spot : October 2004 - February 2005



Objective I : Ensuring crop safety with applied fungicides

Assessments to monitor any possible phytotoxicity symptoms, e.g. scorching, stunting, distortion, were carried out on a range of 12 cultivars of Hebe following the application of 4 fungicide treatments at their normal (N) and twice normal (2N) rates.

No phytotoxicity symptoms were observed in any of the cultivars following 2 applications of the products.

Objective j : Investigating fungicide timing relative to the infection cycle

The fungicide trimming trial conducted during the period July – December 2005 provided additional valuable information to assist growers maintain effective control of leaf-spot with minimum fungicide inputs. An initial disease assessment to investigate the level of leaf spot in the trial plants and to allow for tagging of assessed shoots was carried out on the 4th August. This was followed by a mid-term assessment at the end of September and a final assessment in early December 2005. The data from these assessments is shown in Table 7 and Figure 10.

The initial assessment data effectively represents a baseline level of leaf-spot for comparative treatment effects. The mean number of lesions on the topmost infected leaf pair was scored, along with the number of 'healthy' leaf pairs above the upper most lesions (as shown in Figure 3). No significant differences between the plots in terms of number of lesions, or healthy leaf pairs were apparent at this time. This indicates that the level of infection was even across all the plots prior to the application of fungicides. The overall level of leaf spot at this point in the study was low. By the mid-term assessment, *Stemphylium* leaf spot infection was much more in evidence, with a moderate level of infection particularly in the untreated control plots where the highest incidence of leaf spot was observed. A significant reduction in the incidence of leaf spot was observed with several of the fungicide timing treatments but particularly T2 (fortnightly applications of the tank mix, 4 applications), T3 (monthly applications of the tank mix, 2 applications), T10 (2 applications of the tank mix in September), T11 (2 applications, 1 Signum, 1 Octave both in September), T12 (as T10) & T13 (as T11).

These results show that two applications of either the tank mix, or separate applications of the same products applied singly, are equally as effective as the tank mix applied on a fortnightly basis where four applications had been applied. It also indicates that the products applied singly, as part of an alternating programme were as effective in suppressing the symptoms of leaf spot as two

applications of the products applied as a tank mix. The incidence and severity of leaf-spot was greatest in treatments T7, 8, and 9. These treatments had received the same number and type of application as T11, 12 and 13, the only difference being that T7, 8 and 9 were applied in August rather than in September. Evidence from the spore trapping experiments carried out early in the year suggests that spore release is more prolific during periods of higher temperatures. Maximum daily temperatures for the period of July – November 2005 are shown in Appendix 4. Work by CSL, to demonstrate Koch's postulates and to examine varietal susceptibility indicated that leaf spot lesions developed 2-9 days following infection. The temperature data shows 3 periods where daily maximum temperatures were > 25ºC for a number of days in succession. One such period occurred in mid July. This can possibly be discounted as the main period of spore release as infection initiated then would have been observed on the plants during the 1st assessment at the start of August. Two further periods of warm temperatures occurred at the end of August and the beginning of September. It seems possible that one or both of these occasions resulted in the spore release that initiated the large surge in infection incidence recorded at the end of September. This may also correlate with the comparative degree of disease control that was observed in T10-13 which all received a fungicide treatment on 2nd September, whereas T6-9 received their last application on the 19th August. This study provides some very useful information regarding timing of application with periods of high infection risk.

The final assessment of leaf spot was carried out on the 7th December. The incidence of disease in the untreated control was very severe at this time with an average of 67 lesions/plant above the tag. Only T2 (regular fortnightly sprays with the tank mix) had significantly reduced the level of infection overall. However there was a marked reduction in the disease incidence in the following treatments:-

- T3 (monthly sprays of the tank mix, 4 applications)
- T8 (2 x 2 monthly applications of the tank mix, 4 applications)
- T9 (2 x 2 bi-monthly applications, 2 Signum, 2 Octave, Aug + Oct)
- T10 (2 x 2 bi-monthly applications of the tank mix, 4 applications)
- T11 (2 x 2 bi-monthly application, 2 Signum, 2 Octave, Sept + Nov)
- T12 (2 applications of the tank mix in Sept)
- T13 (1 application each of Signum and Octave in Sept)

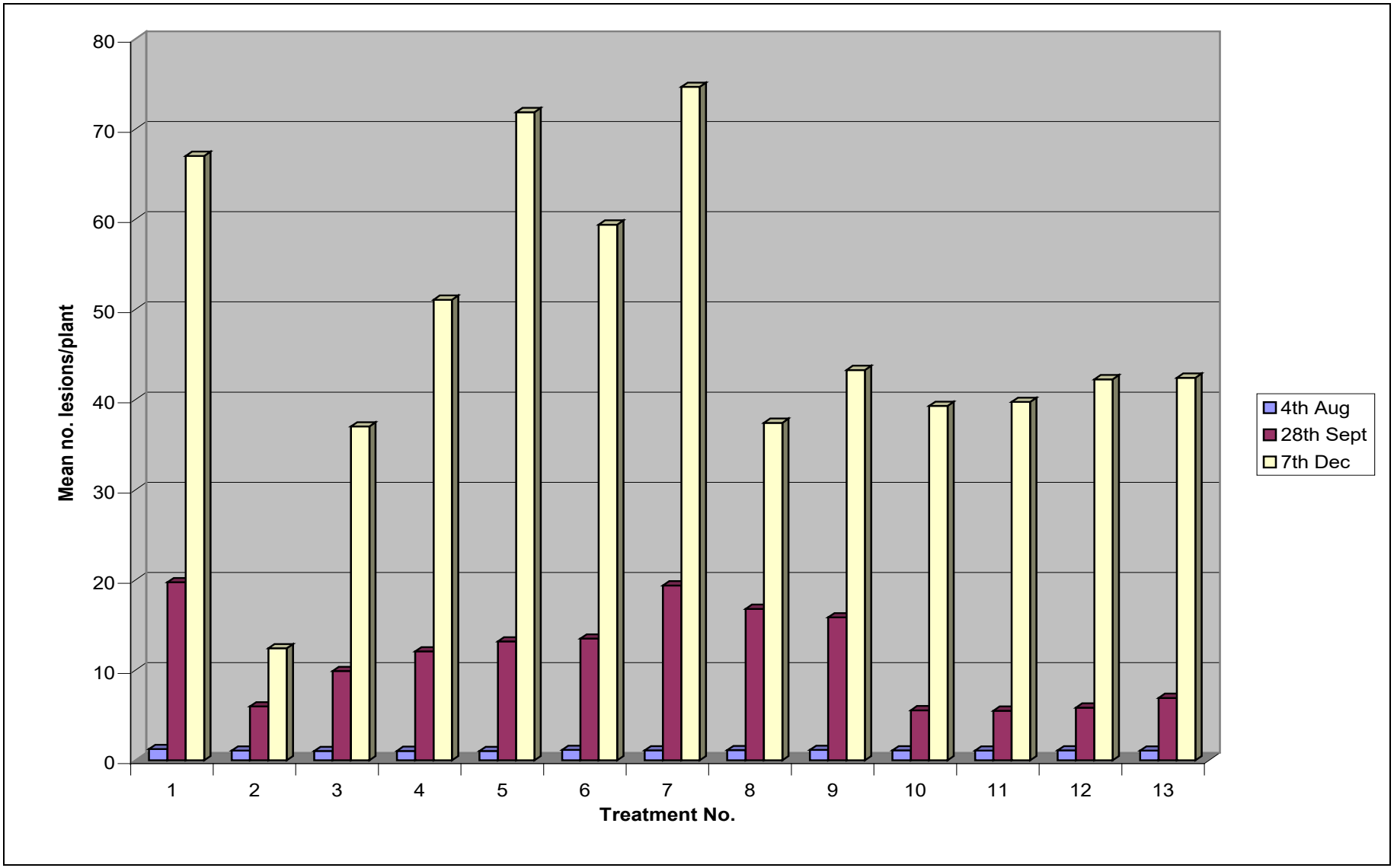
Similar levels of infection were observed across the different treatment regimes listed above and this highlights the fact that the timing, rather than the number of fungicide applications, can be a very important factor in disease control.

Table 7. Comparison of a Range of Fungicide Timings for the Control of Stemphylium Leaf-Spot : August- December 2005

Treatment No.	Mean No. of healthy leaf pairs 4th Aug 05	Mean No. Stemphylium lesion/plant 4th Aug 05	Mean No. of healthy leaf pairs/plant 28th Sept 05	Mean No. Stemphylium lesion/plant 28th Sept 05	Mean No. of total leaf pairs/plant 28th Sept 05	Mean No. of healthy leaf pairs/plant 7th Dec 05	Mean No. Stemphylium lesion/plant 7th Dec 05	Mean No. of total leaf pairs/plant 7th Dec 05
1	5.3^{a}	1.3^{a}	2.4^{ab}	19.8^{a}	13.4^{a}	1.3^{b}	67.0^{a}	14.9^{a}
2	4.1^{a}	1.1^{a}	2.4^{ab}	6.0^{c}	12.5^{a}	2.2^{a}	12.4^{b}	15.9^{a}
3	5.5^{a}	1.0^{a}	2.2^{b}	9.9^{bc}	13.9^{a}	1.7^{ab}	37.0^{ab}	16.3^{a}
4	5.3^{a}	1.1^{a}	2.7^{ab}	12.1^{abc}	13.8^{a}	1.5^{ab}	51.1^{a}	16.9^{a}
5	5.4^{a}	1.0^{a}	2.0^{b}	13.2^{abc}	13.9^{a}	1.2^{b}	71.9^{a}	16.0^{a}
6	4.9^{a}	1.2^{a}	2.5^{ab}	13.5^{abc}	12.8^{a}	1.4^{ab}	59.4^{a}	15.8^{a}
7	4.5^{a}	1.1^{a}	2.5^{ab}	19.4^{a}	13.3^{a}	1.2^{b}	74.7^{a}	14.7^{a}
8	4.7^{a}	1.1^{a}	2.4^{ab}	16.8^{ab}	13.2^{a}	1.6^{ab}	37.4^{ab}	15.6^{a}
9	6.3^{a}	1.2^{a}	2.1^{b}	15.9^{ab}	13.8^{a}	1.3^{b}	43.3^{ab}	16.8^{a}
10	4.7^{a}	1.1^{a}	3.6^{a}	5.6^{c}	12.9^{a}	1.7^{ab}	39.3^{ab}	15.8^{a}
11	5.3^{a}	1.1^{a}	3.1^{ab}	5.5^{c}	13.2^{a}	1.5^{ab}	39.8^{ab}	15.6^{a}
12	5.3^{a}	1.1^{a}	2.8^{ab}	5.9^{c}	13.5^{a}	1.5^{ab}	42.3^{ab}	17.0^{a}
13	5.0^{a}	1.1^{a}	3.5^{a}	6.9^{c}	13.4^{a}	1.7^{ab}	42.4^{ab}	16.7^{a}
LSD (P=0.05)	1.9	0.2	0.7	5.9	1.7	0.5	23.6	2.2
Std. Deviation	1.3	0.1	0.5	4.1	1.2	0.3	16.5	1.5
CV	26.1	12.6	19.1	35.6	8.9	21.3	34.7	9.7

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)

Figure 10. Incidence and severity of *Stemphylium* leaf-spot in the fungicide timing trial : August- December 2005



Objective k : Preparation of an industry fact sheet on leaf diseases of *Hebe*.

A Fact sheet, providing information on the various leaf diseases of *Hebe*, including *Stemphylium* leaf-spot, has been prepared for submission to the HDC.

Discussion

The project was prompted following an initial nursery report of disease control failure in *Hebe*. A severe leaf-spot problem in *Hebe* remained uncontrolled and caused significant crop loss even after repeated application of a conventional fungicide programme for HONS. Initial investigations at STC suggested the causal fungus to be a *Stemphylium* species rather than the more usual *Septoria* spp., which would have been targeted by the conventional fungicide programme. A broader investigation or survey of *Hebe* crops around the country, as a precursor to the main project, quickly established that the *Stemphylium* species was very widespread and damaging to *Hebe* crops and that it was being mis-diagnosed by growers as *Septoria* leaf spot and, accordingly, was being treated with carbendazim e.g. Bavistin routinely.

In the first year of the project we were able to identify the fungus as *Stemphylium solani* (with a 96% confidence limit using molecular techniques) and also demonstrated pathogenicity to *Hebe* through Koch's postulates. The *Stemphylium* sp. therefore appears to be a new, previously unreported, pathogen on this host.

We have also carried out an *in vitro* fungicide screen to provide an early indication of possible fungicides that might provide effective control. This work quickly demonstrated that carbendazim (e.g. Bavistin), which is generally the first choice product for *Septoria* control, to be totally ineffective against the *Stemphylium* sp. and this perhaps suggests that resistance to carbendazim has developed in the pathogen population. However, whilst this is a reasonable assumption, based on the efficacy of carbendazim against similar fungi, it cannot be confirmed without access to baseline isolates not previously exposed to this fungicide. It is, of course, always possible that the fungus has an inherent tolerance to the chemical; though this is considered unlikely as carbendazim is generally reported to be effective in situations where other *Stemphylium* spp. occur as plant pathogens on different hosts.

Those fungicides providing the greatest mycelial inhibition were subsequently tested at STC in a replicated field experiment using infected *Hebe* plants. Several of the experimental, but commercially available, fungicides proved to be very effective and this offers considerable promise for improved disease control in the industry. No symptoms of phytotoxicity were observed following application of the experimental products in this trial. Subsequently, broader crop safety studies on a wider range of cultivars were undertaken, using products demonstrated to be effective against the disease, at their normal (1xN) and twice normal (2xN) application rate. No evidence of

any symptoms of phytotoxicity were observed on the cultivars used in the study. Growers can adjust their spray programmes accordingly, subject to ensuring that the various products are approved for use in their specific situation and safe in the particular environment that they are to be used in.

Epidemiological studies have also been undertaken to investigate the methods of infection, spore survival and dispersal and to identify the optimum conditions for infection and symptom expression. These studies have shown that infection on to *Hebe* cv 'Red Edge' can occur very quickly within 48 hours after inoculation and without the need for a wound or damage to the plant to aid entry. Temperature studies have also indicated that the pathogen has a high temperature optimum for growth (ca. 30^{o}C) and the disease can therefore be expected to be most severe during periods of hot weather. Investigations on the perfect or sexual stage (*Pleospora*) stage of the pathogen have been inconclusive as this spore stage of the fungus was not detected during the period of the study. It had been assumed that this may occur on fallen leaf litter though it was not found on a selection of samples collected from different nurseries. However, it is still advisable to remove as much leaf litter as possible as it potentially still remains an important source for the carry-over (over-wintering) of specific pathogens. The evidence from artificial inoculation studies suggest that none of the Hebe cultivars & types examined were immune from infection, though there were considerable differences in overall susceptibility to the disease. Where possible, growers need to select cultivars and types that have a greater level of tolerance to leaf-spot infection and conversely avoid those that are highly susceptible. Plant breeders need to be alerted to the need to consider tolerance to *Stemphylium* leaf-spot during selection of new types or strains of Hebe.

Studies to investigate the mechanisms, timing and influencing factors in spore dispersal have provided some information though further work would be necessary to draw firm conclusions in this regard. The results of the initial spore trapping experiments carried out during summer 2004 did not indicate a clear diurnal rhythm to conidial (spore) release, although there was some suggestion that more spores were released during the early afternoon than at other times of the day and this may be indicative of a temperature response. Tests carried out in November 2004 to investigate the influence of irrigation/rainfall on spore dispersal were considerably hampered by the almost complete failure of the plants to release spores at this time of the year. Observations in these studies therefore also imply that the temperature influences spore dispersal and suggests that inoculum levels in the environment are likely to be low during cold winter months; and hence disease risk ought to be low. Further spore-trapping experiments carried out in the spring and early summer 2005 supported this hypothesis and provided good evidence to demonstrate increased spore

release during higher temperature periods. This information alone provides valuable data to assist growers in terms of when to apply preventative fungicides to minimise economic crop loss.

The subsequent study on fungicide timing trial undertaken during the late summer and autumn of 2005 provided further information to assist growers in controlling the disease. The trial was able to show that effective control of leaf-spot could be maintained with a reduced number of fungicide applications ideally timed to coincide with high temperature periods when peak spore release can be expected. Whilst it would be necessary to generate more information to refine these initial observations it does provide a good indication that growers could gain a clear financial benefit in terms of reduced fungicide and labour costs for pesticide application.

This project has identified a new leaf-spot disease on Hebe, and has demonstrated that the widely used fungicide carbendazim was ineffective and also identified alternative novel fungicides that are not only efficacious but also safe to use on the crop. The information from the project has been disseminated widely to Hebe growers throughout the duration of the project, predominantly through either one-to-one contact by the research team and/or the HDC grower co-ordinator or via popular articles in the trade press, including HDC News. Early reports from growers who have adopted early recommendations from the project team, especially with respect to fungicide treatments, suggest that crops are much cleaner and the incidence of leaf-spot is much lower and crops are marketable.

Conclusions

- Leaf spot on *Hebe* species is currently a widespread and severe problem in the UK.
- Historically, the predominant leaf-spot on Hebe has been caused by the pycnidial fungus *Septoria exotica*.
- Initial problems reported by a UK grower in 2003 led to preliminary work by STC which indicated that *S. exotica* was not responsible for the leaf-spot and suggested instead that a different fungus, tentatively identified as a *Stemphylium* species, was responsible for the leaf spot.
- Diagnosis using traditional laboratory isolation techniques, backed-up by molecular studies, has suggested that the pathogen responsible for leaf spotting is *Stemphylium solani* (96% confidence limit).
- Koch's postulates have now been successfully undertaken and satisfied; plants inoculated with the *Stemphylium* sp. developed characteristic symptoms from which the same pathogen was re-isolated.
- Carbendazim (e.g. Bavistin) the predominant fungicide used for *Septoria* leaf-spot control on Hebe proved totally ineffective against the *Stemphylium* in *in vitro* tests and that fungicide resistance is suspected. This accounts for why the same fungicide applied routinely for *Septoria* control failed to give adequate control of leaf spot caused by *Stemphylium*.
- *In vitro* screening of a range of experimental, but commercially available, fungicides has provided considerable encouragement as several of the products tested have been found to be effective against *Stemphylium* in agar tests in the laboratory.
- A replicated *in vivo* fungicide trial to evaluate the effectiveness of several candidate products broadly mirrored the results from the *in vitro* laboratory studies and many of the same products provided effective control of *Stemphylium* leaf spot control *in planta*.
- The most effective fungicides in both laboratory and crop studies were pyraclostrobin + boscalid (Signum), iprodione (Rovral), fluazinam (Shirlan) and prochloraz-Mn (Octave). Interestingly, azoxystrobin (Amistar) which did not perform very effectively in agar plate tests did provide good control of the disease *in planta*. This supports results elsewhere which suggest agar plate assays are not particularly reliable for the strobilurin or QoI fungicides. The anilino-

pyrimidine fungicides pyrimethanil (Scala) and mepanipyrim (Frupica), which appeared to provide a moderate-good suppression of *Stemphylium* in the agar plate assay failed to provide effective control of the disease in the trial crop. The remaining triazole products tebuconazole (Folicur), myclobutanil (Systhane), and difenoconazole (Plover), whilst moderately effective in the agar plate assay, were disappointing when used at the crop scale and were less effective than Octave. However, if applied for the control of other diseases in the crop they may provide some suppression of leaf spot.

- Investigations into the relative crop safety of Signum, Rovral, Octave and Amistar at the normal and twice normal application rate on a range of commercially available Hebe cultivars suggested that there were no obvious risks associate with their use on this crop.

- A replicated trial to investigate the optimum timing of fungicide application provided data regarding the most effective number and timing of fungicide applications to control *Stemphylium* leaf spot. The data collected showed that effective control could be maintained by a reduced number of well-timed sprays. Knowledge of the pathogen epidemiology could assist in determining the optimum timing of applications and these should be targeted at high risk periods during peak spore release e.g. periods when temperatures exceed 25ºC. Whilst this information could provide clear economic benefits for commercial growers further refinement would be necessary to ensure robust control under a range of different environmental conditions.

- As the symptoms of *Septoria* and *Stemphylium* leaf-spots are similar yet control measures potentially different growers with a leaf-spot problem are encouraged to secure an accurate identification of the cause by submitting affected leaf material to a commercial diagnostic clinic before implementing control measures.

- Growers with crops confirmed as infected with *Stemphylium* infection should immediately cease use of carbendazim (e.g. Bavistin) and should instead instigate an alternating programme of iprodione (Rovral) and prochloraz-Mn (Octave), subject to crop safety testing on the cultivars being grown under their own local conditions. Further consideration should be made to include other effective fungicides in the programme e.g. boscalid+pyraclostrobin (Signum), subject to meeting the statutory conditions of approval via the Long Term arrangements for Extension of Use.

Technology Transfer

Information collated from the responses to the Grower Survey along with preliminary results from the *in vitro* fungicide screen were circulated to all growers who responded to the survey. An article was also prepared for HDC Project News in June 2004 and there have also been occasional news items relating to the disease in the popular press.

Separately, a brief article on *Hebe* leaf spot was prepared for publication in BSPP News, a popular magazine for members of the British Society of Plant Pathology.

A HDC Fact sheet on leaf diseases of *Hebe* is currently in preparation and will be available in the near future.

Acknowledgements

The assistance of Danny Elliott who was appointed as the HDC Project Co-ordinator & Ian Nelson, Johnsons of Whixley who has provided plants and technical support has been appreciated throughout this study.

Appendices

Appendix 1 : Results from Grower Survey
Appendix 2 : Tabulated data from in vitro resistance tests
Appendix 3 : Tabulated data from fungicide assessments
Appendix 4 : Tabulated temperature records for period of fungicide timing trial.
Appendix 5 : Questionnaire & Information sheet circulated to growers
Appendix 6 : Photographic records of varietal susceptibility to *Stemphylium* in Hebe cultivars

Appendix 1 – Edited results from Grower Survey

Clinic No.	Cultivar	Variegated	Date recd	Severity of spot	Pesticides applied	Source of plants*	Irrigation	Prot/ Outd	1st seen	Results of isolations
PC3323A	Pink Paradise	N	23.03.04	Severe	Aliette/Bav/Bravo (Alt)	C	Overhead	P	Nov/Dec	Stemphylium & Botrytis
PC3323B	Sapphire	N	23.03.04	Moderate	Aliette/Bav/Bravo (Alt)	C	Overhead	P	Nov/Dec	Botrytis & bacteria
PC3323C	Silver Dollar	Y	23.03.04	Mild	Aliette/Bav/Bravo (Alt)	C	Overhead	P	Nov/Dec	*Stemphylium*
PC3327A	Red Edge	N	24.03.04	Severe	Octave, Aliette	C	Overhead	O	3-4yrs	*Stemphylium*
PC3327B	Great Orme	N	24.03.04	Moderate	Octave, Aliette	C	Overhead	O	3-4yrs	*Stemphylium*
PC3327C	Marjorie	N	24.03.04	Mild	Octave, Aliette	C	Overhead	O	3-4yrs	*Stemphylium*
PC3327D	Pinguifolia pagei	N	24.03.04	Moderate	Octave, Aliette	C	Overhead	O	3-4yrs	*Stemphylium*
PC3328A	Albicans	N	24.03.04		Amistar/Aliette/	C	Overhead	O	Spring 2003	*Stemphylium*
PC3328B	Mrs Winder	N	24.03.04	Severe (last winter)	Favour/Sportak	C	Overhead	O	Spring 2003	Stemph, Botrytis & Cylindrocladium
PC3328C	Youngii	N	24.03.04	Severe (last winter)	applied fortnightly	C	Overhead	O	Spring 2003	*Stemphylium*
PC3328D	Rakaiensis	N	24.03.04			C	Overhead	O	Spring 2003	Fusarium, Penic & Cladosporium
PC3329A	Rakaiensis	N	25.03.04	Mild	Amistar/Amistar/Aliette	C	Overhead	O		Stemph, Cladosporium & Botrytis
PC3329B	Pinguifolia pagei	N	25.03.04	Mild	applied every 7-10 days	C	Overhead	O		*Stemphylium*
PC3329C	Autumn Glory	N	25.03.04	Severe		C	Overhead	O		*Stemphylium*
PC3329D	Albicans	N	25.03.04	?		C	Overhead	O		Botrytis & bacteria
PC3330A	Rakaiensis	N	25.03.04	Mild	Aliette fortnightly	C	Overhead	P	6 wks post	*Stemphylium*
PC3330B	Marjorie	N	25.03.04	Mild		C	Overhead	P	potting	*Stemphylium*
PC3330C	Red Edge	N	25.03.04	Moderate		C	Overhead	P		*Stemphylium*
PC3331A	Red Edge BG1	N	25.03.04	Mild	Amistar/Aliette/	BI	Overhead	O	Sep-03	*Stemphylium*
PC3331B	Red Edge LG1	N	25.03.04	Moderate	Fungex/Bravo/Novak	BI	Overhead	O	Mar-04	*Stemphylium*
PC3331C	Red Edge LG2	N	25.03.04	Moderate		BI	Overhead	O	Mar-04	*Stemphylium*
PC3332A	Red Edge	N	25.03.04	Moderate	Fungex & Amistar for small leaf H.	C	Overhead	O	August	Stemph,Cladosporium & *Fusarium*
PC3332B	S. alpina	N	25.03.04	Mild	Aliette/Caramate/Amistar/Favor on	C	Overhead	O	August	*Stemphylium*
	Albicans	N	Not sent	Severe	large leaf *Hebe*s.	C	Overhead	O	August	Not sent
PC3333A	Rakaiensis	N	25.03.04	Severe	Repulse in winter.	C	Overhead	P&O	Aug-02	*Stemphylium*
PC3333B	Red Edge	N	25.03.04	Moderate	Octave at 1st sign	C	Overhead	P&O	Aug-03	*Stemphylium*

PC3333C	Albicans	N	25.03.04	Moderate	of symptoms	C	Overhead	P&O		*Stemphylium*
PC3334A	Pink Paradise	N	29.03.04	Mild	Octave/Bavistin/Bravo/Aliette	BI	Overhead	P	Varies	Botrytis & *Penicillium*
PC3334B	Pink Pixie	N	29.03.04	Mild	Fortnightly rotated sprays	C	Overhead	P		*Stemphylium*
PC3334C	Pink Paradise	N	29.03.04	Mild		C	Overhead	P		*Stemphylium*
PC3334D	Caledonia	N	29.03.04	Mild		C	Overhead	P		*Stemphylium*
PC3337A	Red Edge	N	31.03.04	Mild	Aliette	BI	Overhead	P		*Stemphylium*
PC3337B	Albicans	N	31.03.04	Mild		C	Overhead	P		*Stemphylium*
PC3337C	Nicola's blush	N	31.03.04	Mild			Overhead	P		*Stemphylium* & *Cladosporium*
PC3348A	Pagei	N	2.04.04		Rovral, Scala, Aliette, Chlorothalonil	C	Overhead	P		No identified pathogens
PC3348B	Pagei	N	2.04.04			C	Overhead	P		*Stemphylium*
PC3348C	Red Edge	N	2.04.04			C	Overhead	P		No identified pathogens
PC3348D	Rakaiensis	N	2.04.04			C	Overhead	P		Cladosporium and bacteria
PC3350A	Fran Variegata	Y	05.04.04	Moderate	Compost tea, Aliette, Amistar, Fubol	C	Overhead	P	Autumn	*Botrytis*
PC3350B	Albicans	N	05.04.04	Moderate	Gold, Drench of Octave/Bavistin when	C	Overhead	P	last	*Botrytis*
PC3350C	Silver Dollar	Y	05.04.04	Moderate	spot seen	C	Overhead	P	year	*Stemphylium*
PC3355A	Silver Dollar	N	07.04.04	Severe	Stroby, Bravo 500, Octave, Karamate	C	Overhead	P	Autumn	No identified pathogens
PC3355B	Red Edge	N	07.04.04	Severe	3-4 weekly programme	C	Overhead	P		No identified pathogens
PC3372	Rakaiensis	N	22.04.04	Moderate	Amistar + Repulse throughout season	C C	Overhead	O O	Worse late summer	*Stemphylium*
PC3373A	Pink Paradise	N	22.04.04	Mild	Aliette, Octave, Fubol, Bravo, Fungex		Capillary	P	February	*Stemphylium*
PC3373B	Silver Dollar	Y	22.04.04	Mild			Capillary	P		*Stemphylium*
PC3373C	Caledonia	N	22.04.04	Mild			Capillary	P		*Stemphylium*
PC3393 A	Wiri Dawn	N	04.05.04	Severe	Bavistin, Octave, Repulse, Bravo,	C	Overhead	P	Mid April	*Stemphylium* and other organisms
PC3393B	Pink Elephant	N	04.05.04	Moderate	Chlorothalonil	C	Overhead	P		*Stemphylium*
PC3393C	Just Judy	N	04.05.04	Mild		C	Overhead	P		*Stemphylium*
PC3393D	Valentino	N	04.05.04	Severe		C	Overhead	P		*Stemphylium*

* C = cuttings from own stock, BI = Bought in plants

Appendix 2 – Full data sets from *in-vitro* resistance tests

Active Ingredient	Product	Mean radial growth of fungal mycelium after 3 days (mm)			
		0ppm	2ppm	20ppm	100ppm
azoxystrobin	Amistar	15.4	10.1	6.9	7.0
boscalid + pyraclostrobin	Signum	16.7	0.3	0	0
carbendazim	Bavistin	19.4	18.7	17.9	15.8
chlorothalonil	Bravo 500	17.1	17.7	17.0	16.3
iprodione	Rovral WP	25.1	0	0	0
fluazinam	Shirlan	16.7	1.9	0.7	0
mancozeb	Karamate	25.1	26.0	15.4	9.6
mepanipyrim	Frupica	19.4	12.6	7.5	6.6
myclobutanil	Systhane	19.4	9.5	3.8	0
Prochloraz-Mn	Octave	25.1	11.9	2.9	0
pyrimethanil	Scala	17.1	5.0	2.1	0
tebuconazole	Folicur	17.1	9.1	3.9	0
tolylfluanid	Elvaron Multi	16.7	10.5	6.6	5.0

Appendix 3 – Field Fungicide Efficacy Trial – tables of results

Table 1a. Baseline disease assessment – 14 September 2004

Treatment	Mean number of leaf pairs/plant	Mean number of disease lesions/plant
Water control	2.0 (2.02)	1.7 (2.24)
Bavistin	1.9 (2.25)	1.5 (1.56)
Rovral WP	1.5 (1.88)	1.6 (1.70)
Signum	2.1 (3.17)	1.8 (2.84)
Shirlan	2.3 (2.42)	1.6 (2.00)
Scala	2.0 (2.46)	1.5 (1.62)
Octave	2.1 (2.54)	1.5 (1.76)
Folicur	2.6 (3.88)	1.6 (2.03)
Plover	2.1 (3.06)	1.3 (1.31)
Systhane	1.8 (2.01)	1.7 (2.02)
Amistar	1.9 (1.79)	1.8 (2.05)
Frupica	1.8(2.20)	2.2 (3.2)

Numbers in brackets indicate standard deviation

Table 2a. Disease assessment 18 October 2004

Treatment	Total number of leaf pairs	Number of healthy leaf pairs	Mean number of disease lesions
Water control	4.4 (2.47)^{a}	3.0 (2.91)^{a}	1.4 (3.31)^{a}
Bavistin	4.4 (2.54)^{a}	2.9 (3.39)^{a}	1.1 (2.71)^{abc}
Rovral WP	4.4 (2.10)^{a}	3.9 (2.98)^{a}	0.3 (1.05)^{e}
Signum	4.5 (2.07)^{a}	4.2 (2.18)^{a}	0.3 (1.17)^{e}
Shirlan	4.9 (2.85)^{a}	4.1 (3.02)^{a}	0.6 (2.15)^{bcde}
Scala	4.8 (2.78)^{a}	3.1 (3.33)^{a}	1.3 (3.50)^{ab}
Octave	4.6 (2.23)^{a}	4.0 (2.86)^{a}	0.4 (1.40)^{de}
Folicur	5.5 (3.84)^{a}	3.4 (3.97)^{a}	1.6 (4.25)^{a}
Plover	4.7 (2.98)^{a}	3.3 (2.93)^{a}	1.1 (2.85)^{abcd}
Systhane	4.7 (2.30)^{a}	3.2 (3.15)^{a}	1.3 (2.71)^{a}
Amistar	4.5 (1.45)^{a}	3.7 (2.40)^{a}	0.5 (2.00)^{cde}
Frupica	4.5 (1.96)^{a}	3.2 (2.84)^{a}	0.9 (1.88)^{abcd}

The figures in brackets indicate the standard deviation from the mean.
Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls).

Table 3a. Disease assessment – 15 November 2004

Treatment	Total number of leaf pairs	Number of healthy leaf pairs	Mean number of disease lesions
Water control	5.3 (2.22)^{a}	2.8 (3.88)^{cde}	2.6 (5.07)^{ab}
Bavistin	5.2 (2.61)^{a}	2.0 (3.64)^{e}	3.4 (6.68)^{a}
Rovral	5.4 (2.20)^{a}	3.3 (4.26)^{bcde}	1.3 (2.60)^{bc}
Signum	5.8 (3.01)^{a}	4.9 (3.81)^{a}	0.3 (1.08)^{d}
Shirlan	5.7 (3.69)^{a}	4.7 (3.63)^{ab}	1.1 (3.88)^{cd}
Scala	5.7 (2.85)^{a}	2.1 (3.72)^{e}	3.3 (6.79)^{a}
Octave	5.5 (2.43)^{a}	3.7 (4.39)^{abcd}	0.8 (1.79)^{cd}
Folicur	6.2 (3.49)^{a}	2.7 (4.23)^{cde}	3.4 (8.01)^{a}
Plover	5.5 (2.21)^{a}	3.1 (4.51)^{cde}	2.0 (3.86)^{abc}
Systhane	5.7 (1.86)^{a}	2.9 (4.07)^{cde}	2.9 (4.58)^{a}
Amistar	5.4 (1.48)^{a}	4.1 (2.95)^{abc}	1.1 (3.67)^{cd}
Frupica	5.4 (2.01)^{a}	2.4 (4.46)^{de}	2.7 (6.13)^{ab}

The figures in brackets indicate the standard deviation from the mean.
Figures in the same column followed by a different letter significantly differ (P=0.05)

Table 4a. Disease assessment – 4 February 2005

Treatment	Total number of leaf pairs	Number of healthy leaf pairs	Mean number of disease lesions
Water control	6.9^{a}	3.6^{b}	3.4^{a}
Bavistin	6.5^{a}	3.1^{b}	3.0^{a}
Rovral	6.6^{a}	4.8^{ab}	1.1^{ab}
Signum	7.0^{a}	6.3^{a}	0.3^{b}
Shirlan	7.4^{a}	6.3^{a}	0.7^{ab}
Scala	7.1^{a}	3.6^{b}	2.9^{a}
Octave	7.0^{a}	4.9^{ab}	0.8^{ab}
Folicur	7.2^{a}	3.5^{b}	3.0^{a}
Plover	7.3^{a}	4.7^{ab}	2.3^{ab}
Systhane	7.2^{a}	3.8^{b}	3.0^{a}
Amistar	6.7^{a}	5.1^{ab}	1.1^{ab}
Frupica	7.0^{a}	3.9^{b}	2.4^{ab}

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)

Appendix 4 – Maximum daily temperatures during period of the fungicide timing trial

Month / Date	July	August	September	October	November
	Max (ºC)	Max (ºC)	Max (ºC)	Max (ºC)	Max (ºC)
1	22.3	19.4	29.4	21.5	13.1
2	25.0	24.6	29.4	15.4	17.5
3	24.0	21.0	21.4	14.4	12.0
4	16.5	21.5	25.6	14.9	11.1
5	16.9	21.8	25.0	16.7	15.0
6	19.6	20.9	25.5	13.9	14.5
7	20.5	19.1	22.4	16.4	12.7
8	19.6	23.5	21.4	15.9	15.4
9	25.4	22.0	19.3	ko14.3	14.3
10	28.0	24.0	15.6	19.4	15.2
11	26.8	24.7	17.2	27.0	14.7
12	26.8	20.2	22.0	20.0	11.1
13	27.9	19.0	21.2	13.9	8.5
14	27.9	19.9	20.0	14.8	13.0
15	23.0	22.0	14.5	16.7	10.6
16	23.2	19.0	14.1	17.6	6.6
17	28.0	26.9	16.3	15.0	6.0
18	22.1	26.6	18.8	13.5	6.0
19	20.2	21.7	19.7	13.5	6.9
20	22.0	22.4	18.9	14.9	8.4
21	22.0	24.6	19.4	15.4	7.7
22	20.6	24.4	21.5	11.6	4.4
23	20.2	21.1	18.1	11.7	9.5
24	17.0	18.5	15.2	17.0	9.0
25	17.0	18.5	19.4	15.2	4.8
26	18.8	16.2	17.4	16.4	5.8
27	20.0	19.7	18.0	19.0	4.7
28	18.5	21.9	15.7	17.4	3.4
29	19.5	21.4	15.1	16.7	4.5
30	18.0	25.2	21.6	17.4	7.1
31	18.9	29.4		13.7	
Total	676.8	681.1	599.1	501.2	293.5
Mean	21.8	21.9	19.97	16.17	9.78
Highest max.	28.0	29.4	29.4	27.0	17.5
Lowest max.	16.5	16.2	14.1	11.6	3.4

Appendix 5 – Questionnaire circulated to Growers

Name …….…….	Nursery Address …….
Tel ……..…….	…….
Mob ……..……..	…….
Fax ……..……	
Email ……..……	Postcode……

Name of Cultivars sampled	Leaf spot severity (tick one/cv) **Severe**	**Moderate**	**Mild**
1……..	☐	☐	☐
2 …….	☐	☐	☐
3 …….	☐	☐	☐
4 …….	☐	☐	☐

Fungicide Programme
Please specify details of the fungicide programme applied to your *Hebe* stock

Source of Stock

Were the plants:
- Bought in as mature plants ☐
- Bought in as cuttings/young plants ☐
- Cuttings taken from own mother stock ☐

Cultural Practice

Is the irrigation overhead ☐ capillary ☐ drip ☐ ? (please tick)

Are the plants protected ☐ or outdoors ☐? (please tick)

Rate of controlled release fertilizer in compost ☐ kg/m^3

When was the leaf spot first seen? …….

Do you know what is causing the leaf spot? ……

Have you taken measures to get the problem identified? ……

Do you use diagnostic services routinely? ……..

Note: All results of isolation tests relating to these samples will remain confidential. If you wish to know the results specifically relating to your nursery please tick this box. ☐

Information sheet sent to Growers

Grower Study on Leaf spots of *Hebe*
(HDC Project : HNS 128)

SAMPLE REQUEST

- **As part of the above project we would like to determine the incidence of leaf-spot pathogens in Hebe cultivars across the country.**
- **Please check your stock for leaf-spot symptoms (see photo's below)**
- **Where leaf-spot symptoms are found collect a few shoots and place in the bags provided. If the symptoms are different don't worry; send them anyway.**
- **Where possible, we would like samples from stock plants, rooted cutting and from plants grown on for sale (protected and/or outdoors). Please mark the bags accordingly.**
- **Select samples from a maximum of 3 different cultivars per nursery.**
- **Please spare a few minutes and provide the information on the form included.**
- **Place all the samples collected on the nursery in the pre-paid padded envelope and**

- The information will be used to better focus the project on the predominant diseases and their control.
- All information relating specifically to what we find on your nursery will be kept in strict confidence and will not be divulged to third parties without your prior permission.
- An industry Factsheet will be produced at the end of the project to assist growers identify and control leaf-spots on Hebe more effectively.

Appendix 6 : Photographic records of varietal susceptibility following inoculation with *Stemphylium* at CSL

Plate 12. Comparison of variety Albicans inoculated with mixed isolates of *Stemphylium*

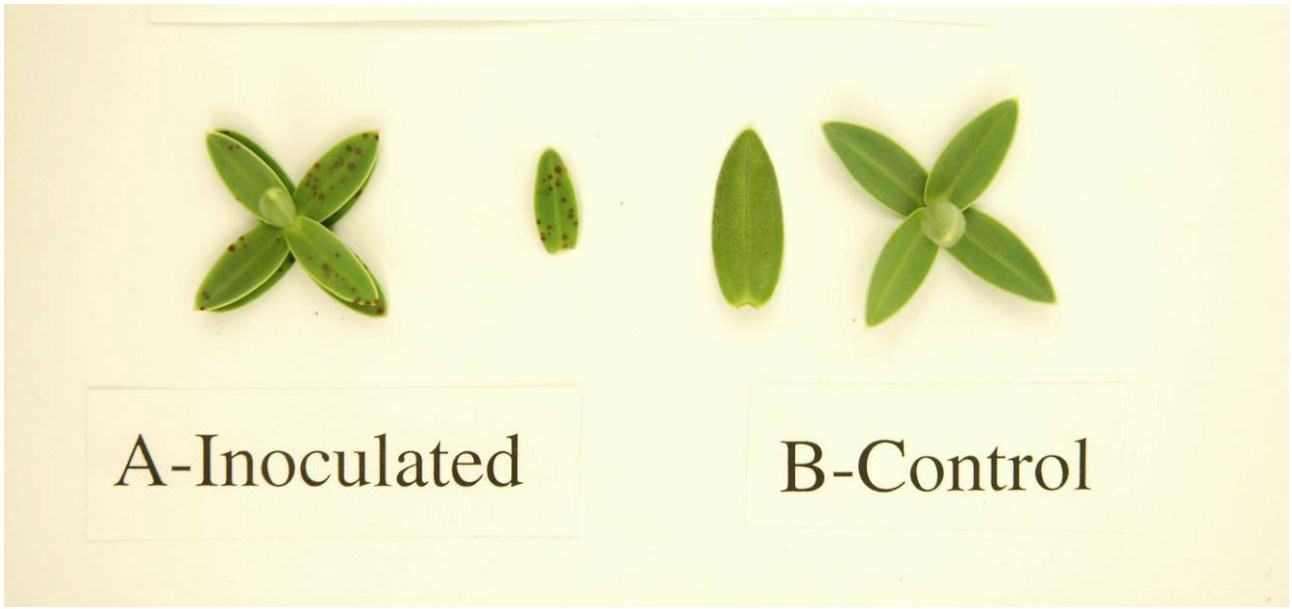


Plate 13. Comparison of variety Baby Marie inoculated with mixed isolates of *Stemphylium*

Plate 14. Comparison of variety Champion inoculated with mixed isolates of *Stemphylium* Champion

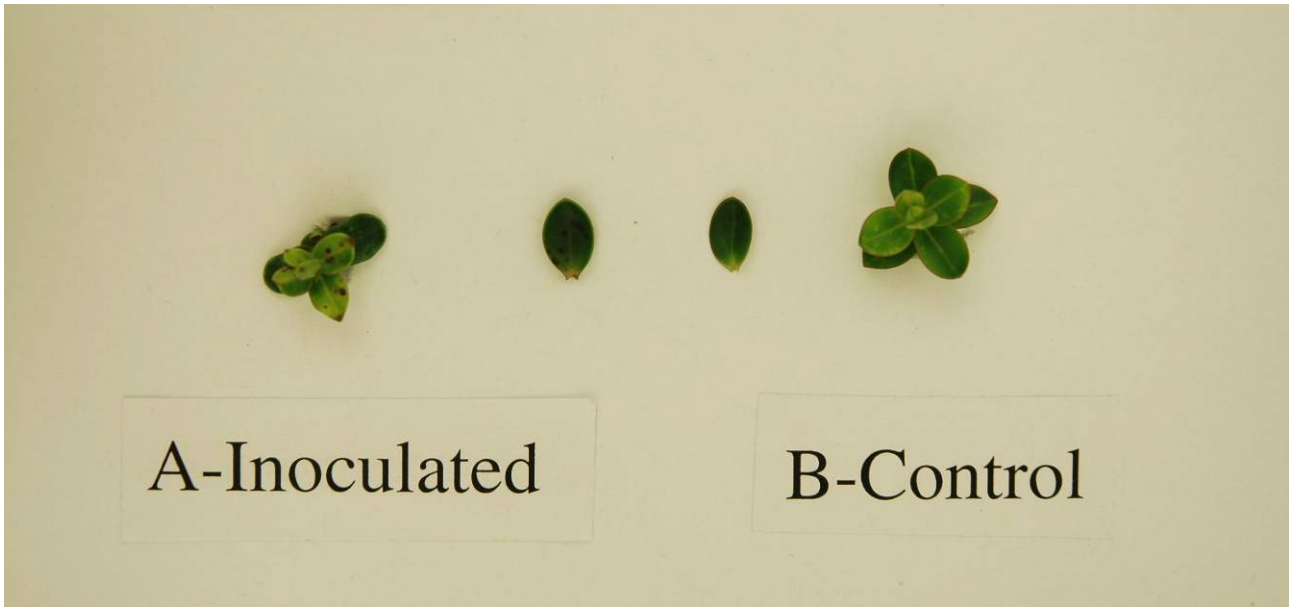


Plate 15. Comparison of variety Pageii inoculated with mixed isolates of *Stemphylium* Pageii

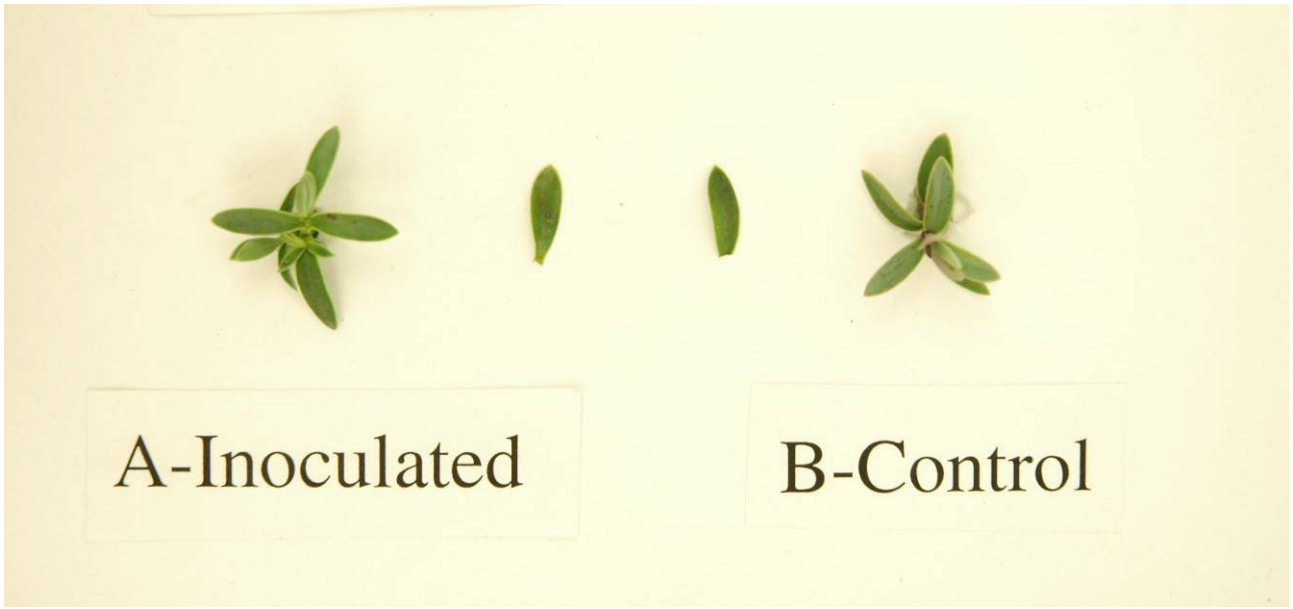


Plate 16. Comparison of variety Rakaiensis inoculated with mixed isolates of *Stemphylium*

Plate 17. Comparison of variety Red Edge inoculated with mixed isolates of *Stemphylium*

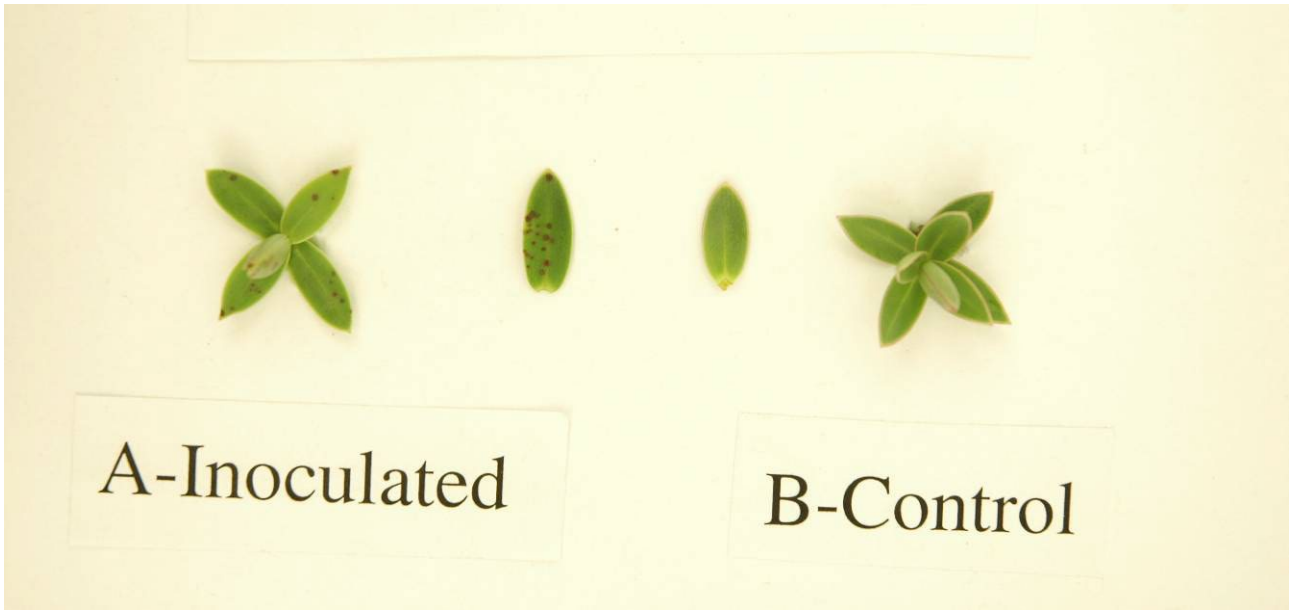


Plate 18. Comparison of variety Silver dollar inoculated with mixed isolates of *Stemphylium*

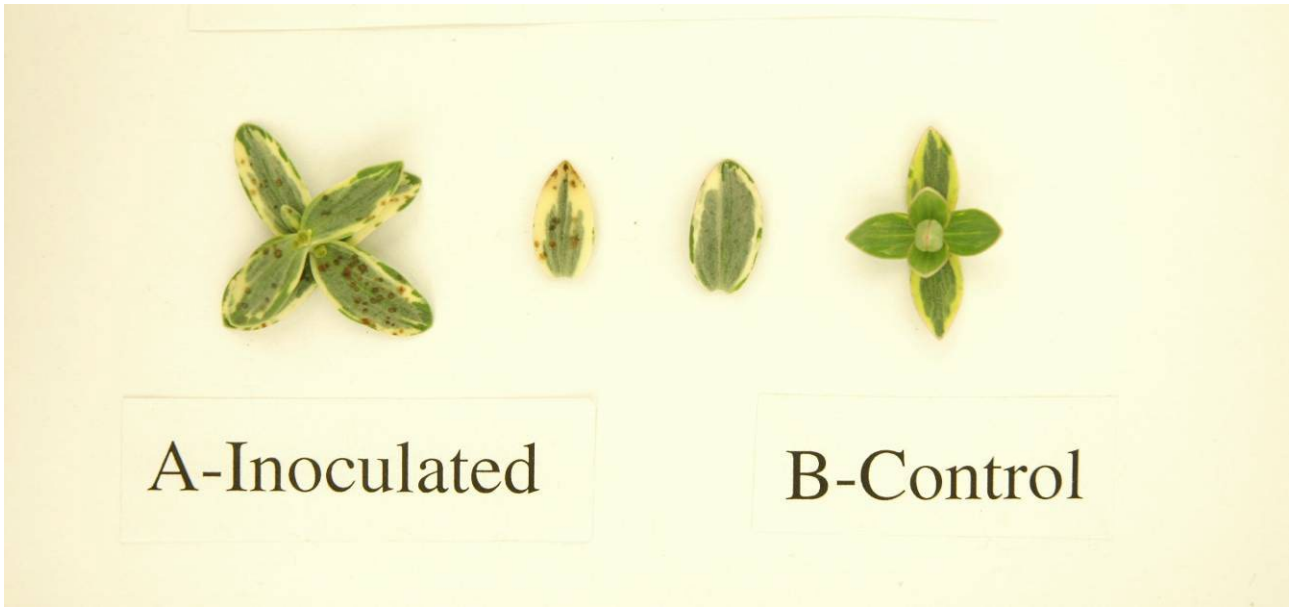


Plate 19. Comparison of variety Sweet Kim inoculated with mixed isolates of *Stemphylium*

Plate 20. Comparison of variety Vernicosa inoculated with mixed isolates of *Stemphylium*

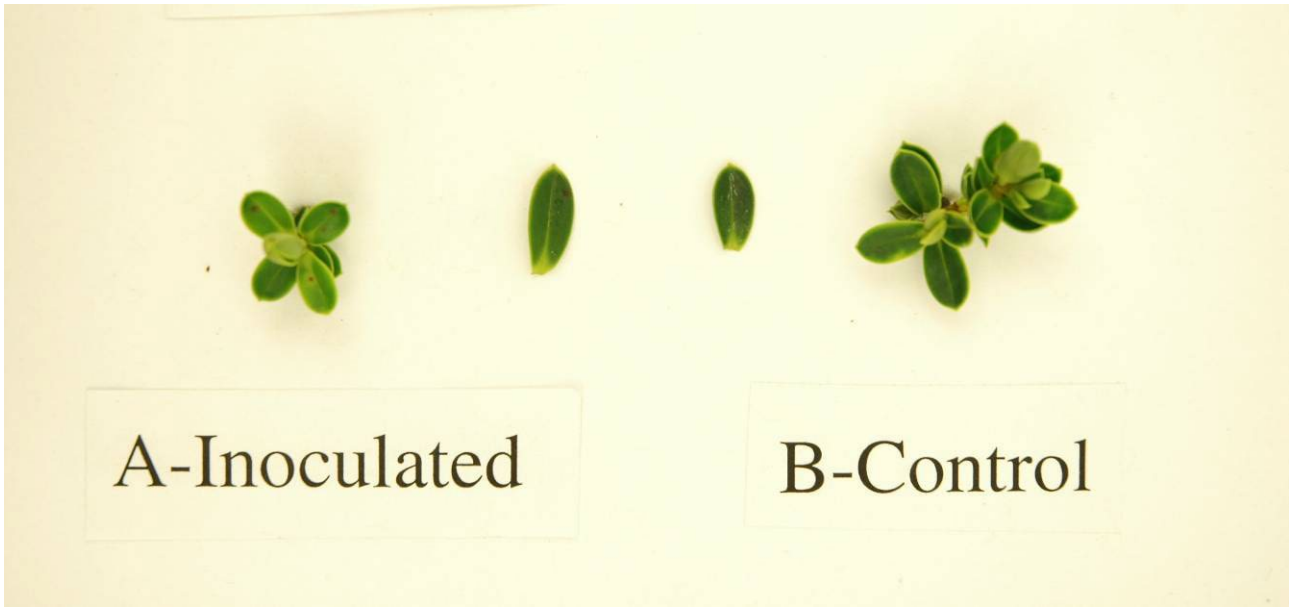


Plate 21. Comparison of variety Wirimist inoculated with mixed isolates of *Stemphylium*