Project Title:	Investigation into the epidemiology and control of *Stemphylium* (*Pleospora*) leaf spot of *Hebe*.
Project Number:	HNS 128
Project Leader:	Dr G M McPherson MBPR (Hort.) Technical Director Crop Protection Services Stockbridge Technology Centre Cawood, Selby North Yorkshire YO8 3TZ
Key Workers:	Ms C Lambourne, Dr S Parker
Report:	Annual Report Year 1, March 2005
Location:	STC Ltd. Cawood, Selby North Yorkshire YO8 3TZ
Project Co-ordinator:	Mr D Elliott HONS Nursery Business Advisors 2 Albemarle Road York YO23 1FB
Date Commenced:	February 2004
Due completion date:	March 2006
Keywords:	*Hebe*, leaf spot, *Stemphylium solani*, *Pleospora*, disease control, fungicides, crop safety, efficacy.

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

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

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CONTENTS

GROWER SUMMARY

Headlines

This work has established that:

- *Hebe* leaf spot is a widespread problem primarily caused by a fungus from the *Stemphylium* group and not the previously recorded fungus *Septoria exotica*.

- In a field trial on *Hebe* 'Red Edge', the fungicides Signum, Rovral, Shirlan, Octave and Amistar proved the most effective in preventing new infections on young shoots. Bavistin was totally ineffective and the pathogen was found to be totally resistant to this fungicide. Systhane was relatively ineffective as were Scala and Frupica.

Background and Expected Deliverables

Until recently, leaf spots occurring on *Hebe* cultivars were generally regarded to be due to the fungus *Septoria exotica*, a well recognised leaf spot pathogen on this host, and treated with carbendazim (eg Bavistin) accordingly. However, on some nurseries, control of *Hebe* leaf spot was 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 STC (plate 1). Preliminary investigations on samples from the nursery were unable to detect *Septoria* spp. associated with the leaf spots. Instead a different fungus, tentatively identified as *Stemphylium* sp., was isolated consistently. It quickly became clear that the standard fungicide programme deployed by this particular nursery was having little or no effect in controlling the problem.

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

Broader enquiries during 2003 indicated that leaf spotting on *Hebe* was more widespread and anecdotal evidence suggested that losses on some nurseries were exceptionally large. Without an effective understanding of the disease on HONS species, specifically how it is disseminated, what spore forms are produced, how it over winters, and the best control

strategies to use, it was recognised that plants would continue to be infected and financial losses incurred. A more detailed knowledge of the fungus would certainly help to identify appropriate cultural control measures, select potential fungicides and target their application (method, frequency and timing). Dissemination of such information to the industry via a HDC factsheet at the end of this project should lead to an improvement in plant quality and hence to a reduction in financial losses due to the disease.

Summary of the Project and Main Conclusions

Before embarking on a 2 year project it was agreed that it was necessary to establish the full extent of the problem in the industry. Therefore an information sheet and questionnaire was circulated to a broad range of *Hebe* growers (28 in total), following consultation with the Project Co-ordinator, in early Spring 2004. This included a request for samples to be sent to the Plant Clinic at STC. Responses from the survey were good with 18 growers replying (64% response rate) and a great deal of information regarding the incidence and perceived cause of leaf spots on *Hebe* was gathered.

It was apparent that the majority of *Hebe* growers were experiencing significant problems with leaf spotting, indeed two growers had stopped growing *Hebe* altogether due to problems in producing quality plants. Many of those who responded assumed the leaf spot to be caused by *Septoria*. Nearly all growers linked the disease with specific temperature and humidity conditions. Approximately 50 samples of *Hebe* with leaf spot symptoms were forwarded to the STC laboratory for diagnosis. Independent tests by both STC and CSL identified *Stemphylium* as the causal agent of the leaf spot in 74% of these samples. Interestingly, no *Septoria* was seen at all on these samples.

As a result of these initial findings the 2-year project was commissioned to gain a better understanding of this new disease on *Hebe*. During 2004, CSL focused mainly on confirming the presumptive identification of the causal agent of the leaf spot symptom on *Hebe* made by STC and also on various epidemiological aspects of the disease. STC focused on investigations relating to pathogen control using alternative fungicides, so that timely information and advice could be provided as a basis for growers to adjust disease control strategies.

A summary of progress on the project objectives is outlined below:-

Objective a : Isolation and Identification of the pathogen

From the leaf spot samples submitted by growers in Spring 2004 a *Stemphylium* sp. was isolated consistently. Using modern molecular techniques deployed at CSL a 96% match to *Stemphylium solani* was secured.

Objective b : Confirming pathogenicity

Investigations into the pathogenicity of the *Stemphylium* sp. have also been carried out by CSL. The popular and highly susceptible cultivar 'Red Edge' was used in the tests. Inoculation studies have shown that a spore suspension of *Stemphylium* was able to infect previously healthy leaves and produce characteristic lesions within 2 days. Infection does not appear to depend on the presence of leaf damage. Infection occurred equally well on leaves that were wounded or unwounded prior to inoculation and the fungus could readily be re-isolated.

Objective c : Examining spore dispersal

Studies to investigate the primary means of spore dispersal of the pathogen have been carried out at STC. Early indications are that spores (conidia) are liberated from infected crops into the air. Spore numbers are not particularly high and don't appear to show a strong diurnal rhythm, though there is a suggestion of greater spore release in the afternoon. Spore liberation appears to be minimal during the winter months, which might be indicative of some temperature

dependence for disease progress. Further work to understand dispersal processes are planned for Spring-Summer 2005.

Objective d : Studying infection conditions for the *Stemphylium* sp.
Laboratory inoculation studies are being carried out to determine the optimum temperature for growth of the fungus and the potential of the two spore types (ascospores & conidia) to infect plants. This work is still underway and will be reported on later.

Objective e : Investigating the role of leaf litter in disease epidemiology
The role played by leaf litter around over-wintering *Hebe* plants has been investigated by regularly collecting debris from untreated plants. To date the fungus has not been found in the leaf litter collected to date and the risk remains undetermined at this stage. Further study of this area is planned during 2005.

Objective f : Studying varietal susceptibility to the disease
This work has been re-scheduled to be undertaken in Spring 2005.

Objective g : Laboratory Fungicide Screening
A selection of isolates collected from different parts of the UK during the survey were tested in the laboratory to investigate the relative sensitivity of the pathogen to 13 fungicides. 13 fungicides were tested representing a wide range of Modes of Action (MoA). They included industry standards eg carbendazim, iprodione and pyrimethanil (as used in some HONS species for *Botrytis* control) along with other commercially available fungicides such as mepanipyrim (Frupica), tebuconazole (Folicur) and boscalid + pyraclostrobin (Signum).

The laboratory studies suggested that iprodione, boscalid + pyraclostrobin and fluazinam all provided significant reductions in the amount of mycelial growth on agar at 2ppm. Pyrimethanil, prochloraz, myclobutanil & tebuconazole also showed some promise in this laboratory based test.

These data have therefore identified some key fungicides to take forward into field trials and it also provide base-line measurements of the pathogen's sensitivity to the active ingredients before they are used regularly on commercial holdings. These baseline measures will provide an important benchmark for any future monitoring of sensitivity shifts in the pathogen to specific active ingredients.

Objective h : Fungicide comparisons in field trials
A large-scale replicated field trial was carried out at STC in Autumn 2004 using *Hebe* cv. Red Edge to investigate the efficacy of a short-list of fungicides to control *Stemphylium* leaf spot. Eleven fungicides were finally selected for the trial, again covering a range of industry standards and other commercially available products that had performed well during the preliminary laboratory studies.

The results of this work largely 'mirrored' those seen with the laboratory work, though there were a few exceptions. The laboratory study using azoxystrobin indicated that the chemical produced only moderate reductions in mycelial growth even at 100ppm, whilst in the field it resulted in significantly fewer disease lesions than the untreated control and it proved to be one of the more effective products. It is documented elsewhere that laboratory agar studies with strobilurin (or QoI) fungicides do not necessarily reflect their performance in the field accurately; and this probably relates to the mode of action on the host tissues. These results also highlight the importance of field studies to quantify fully the performance of fungicides; laboratory based screens provide a method to determine suitable candidates for more detailed work.

In the field study the greatest level of disease suppression was seen in the plants treated with Signum (boscalid + pyraclostrobin). Compared to the untreated control, Rovral (iprodione) and Shirlan (fluazinam) also reduced the level of disease significantly. The aniline-pyrimidine

fungicides Scala (pyrimethanil) & Frupica (mepanipyrim) and several of the triazole fungicides Folicur (tebuconazole), Systhane (myclobutanil) & Plover (difenoconazole) performed relatively poorly in the field study even though several of the products tested in the laboratory appeared to provide moderate-good inhibition of the *Stemphylium* fungus on agar. Overall, however, these results are extremely encouraging and ought to allow the industry to adjust their spray programmes to take account of this new situation and hopefully regain effective control of the new aggressive leaf spot on *Hebe*.

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, eg 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.

Objective I : Ensuring crop safety with applied fungicides
This component of the project is scheduled to be undertaken in late Spring - early Summer 2005.

Objective j : Investigating fungicide timing relative to the infection cycle
This component of the project is scheduled to be undertaken in late Summer 2005.

Objective k : Preparation of an industry factsheet on leaf diseases of *Hebe*
A factsheet, which will provide information on the various leaf diseases of *Hebe*, including *Stemphylium* leaf spot, will be prepared towards the end of the project in early Spring 2006.

Financial Benefits

The financial benefits from the first year of this project are already being realised in the HONS sector. The provisional information on the disease has already allowed growers to adjust their spray programmes and early indications are that control of the leaf spot has already been improved on some nurseries.

Towards the end of the project, a grower factsheet will be produced outlining the results of the project and providing additional information on other leaf diseases on this crop.

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. Our survey has found no incidence of *Septoria*.
- Be aware that a new pathogen caused by a *Stemphylium* sp. is causing extensive and often severe leaf spotting on a large range of *Hebe* cultivars. Where leaf spot symptoms are found ensure accurate identification via 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 and consider regular removal of leaf debris from around infected plants as a precaution against potential carry-over of disease.
- Where leaf spot is a problem on *Hebe* review your spray schedule and do not rely on the use of carbendazim (eg 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.

- Be aware that the work to evaluate varietal crop safety with the novel fungicides has not yet been undertaken and that use of any new products will be at 'growers own risk'.
- Keep abreast of developments in this project through regular contact with the Project Coordinator, other industry consultants, the STC Project Manager, HDC or CSL.

SCIENCE SECTION

Introduction

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

plate 2. Characteristic leaf spot symptoms of *Septoria exotica* lesions on *Hebe*

* photo courtesy of Dr T M O'Neill, ADAS

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 3 : Characteristic symptoms of *Stemphylium* leaf spot on *Hebe* cv. Red Edge

plate 4 : Characteristic growth of *Stemphylium* sp. isolated from *Hebe* on agar

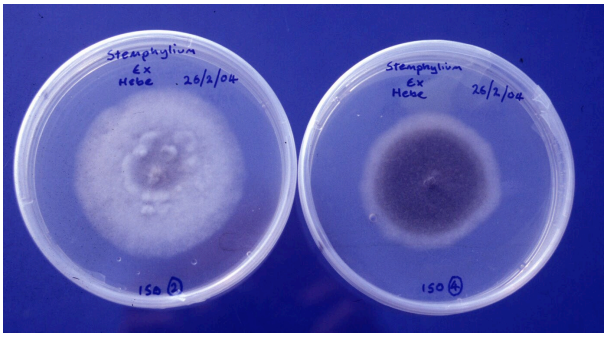
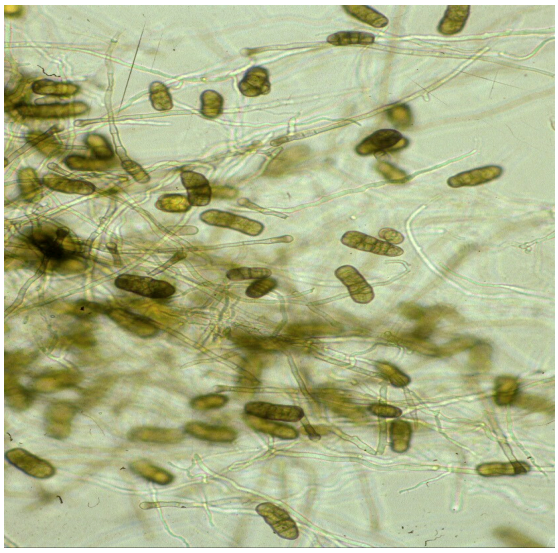


plate 5 : Characteristic spores of *Stemphylium* sp. isolated from *Hebe* on agar



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 have been 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. Morphological investigations into the identity of the organism are also in progress at CSL.

Objective b : Demonstrating Koch's postulates

Stemphylium spores produced from Tap Water Agar cultures were harvested and diluted to a final concentration of 1 x 10^5 - 1 x 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, and will continue in 2005. Initial experiments using an exposed agar plate method of spore trapping were carried out in July 2004 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 on top of the crop 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.

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 left unwatered at this time. 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.

This work has been re-scheduled to be undertaken in April 2005.

Objective e : Investigating the Role of Leaf Litter in Disease Epidemiology

Leaf litter samples collected from commercial nurseries were examined for presence of the sexual stage of the pathogen.

Objective f : Studying Varietal Susceptibility to the Disease

This work has been re-scheduled to be undertaken in Spring 2005.

Objective g : *In Vitro* Fungicide Screening

An initial *in vitro* screen of fungicides was carried out at STC during 2003 using 5 isolates of *Stemphylium* all collected from a severely infected batch of plants supplied to the STC Plant Clinic by a concerned grower. These tests were subsequently repeated using 5 isolates of *Stemphylium* collected from infected *Hebe* plants submitted during the HDC grower survey. 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	Geographical location
1	PC 3323C	Silver Dollar	Wales
2	PC 3348b	Pagei	Yorkshire
3	PC 3350c	Silver Dollar	Leicestershire
4	PC 3372	Rakaiensis	Middlesbrough
5	PC 3373c	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 plates and the unamended plates (0ppm). PDA was used throughout. Plugs (5mm diameter) from 3-4 day old cultures of *Stemphylium* were placed centrally on 90cm Petri-dishes of 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

Objective h : *In Vivo* Fungicide Comparisons

A batch of *Hebe* cv. Red Edge which had a moderate-severe infection with *Stemphylium* leaf spot were arranged in a randomised plot layout consisting of 12 treatments, each with 4 replicates. For each treatment, 2 plots were composed of 20 small *Hebe* plants, and 2 plots of 15 large *Hebe* plants (35 plants/plot in total). 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 (diagram 1).

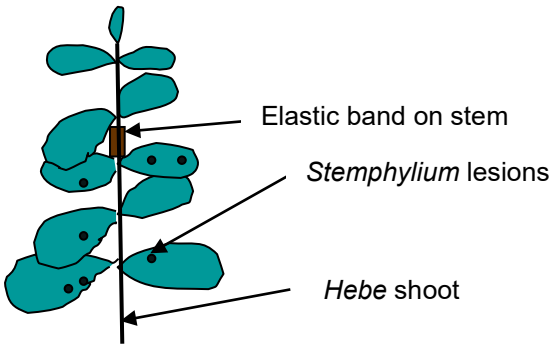


Diagram 1: Schematic diagram of a *Hebe* shoot with an elastic band affixed between infected and uninfected tissue.

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 runoff.

Table 3: Details of the 12 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	1.80 kg/ha	250
5. Shirlan*	Fluazinam*	1.50 l/ha	250
6. Scala	Pyrimethanil	0.1 g/l	250
7. Octave	Prochloraz	2 g/l	250
8. Folicur	Tebuconazole	1 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 September 2004	Replicated *Hebe* trial laid out
14 September	Baseline disease assessment carried out
16 September	1st Fungicide application
5 October	2nd Fungicide application
18 October	Disease Assessment
18 October	3rd Fungicide application
4 November	4th Fungicide application
15 November	Disease assessment
4 February 2005	Final disease assessment

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 (diagram 2).

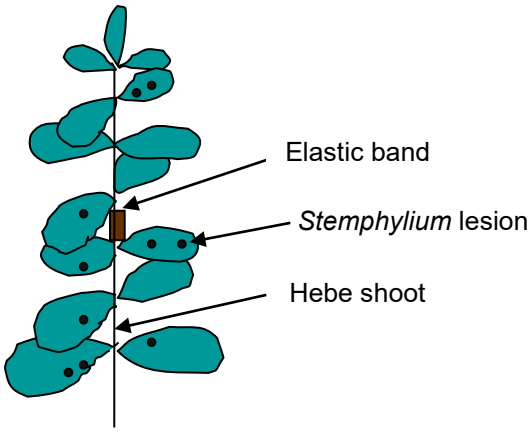


Diagram 2: Schematic diagram of a *Stemphylium*-infected *Hebe* shoot. The shoot score: total number of leaf pairs = 4; number of healthy leaf pairs from the apex = 1; and, the number of disease lesions = 3.

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
This component of the project is scheduled to be undertaken in late Spring - early Summer 2005.

Objective j : Investigating Fungicide Timing relative to the Infection Cycle
This component of the project is scheduled to be undertaken in late Summer 2005.

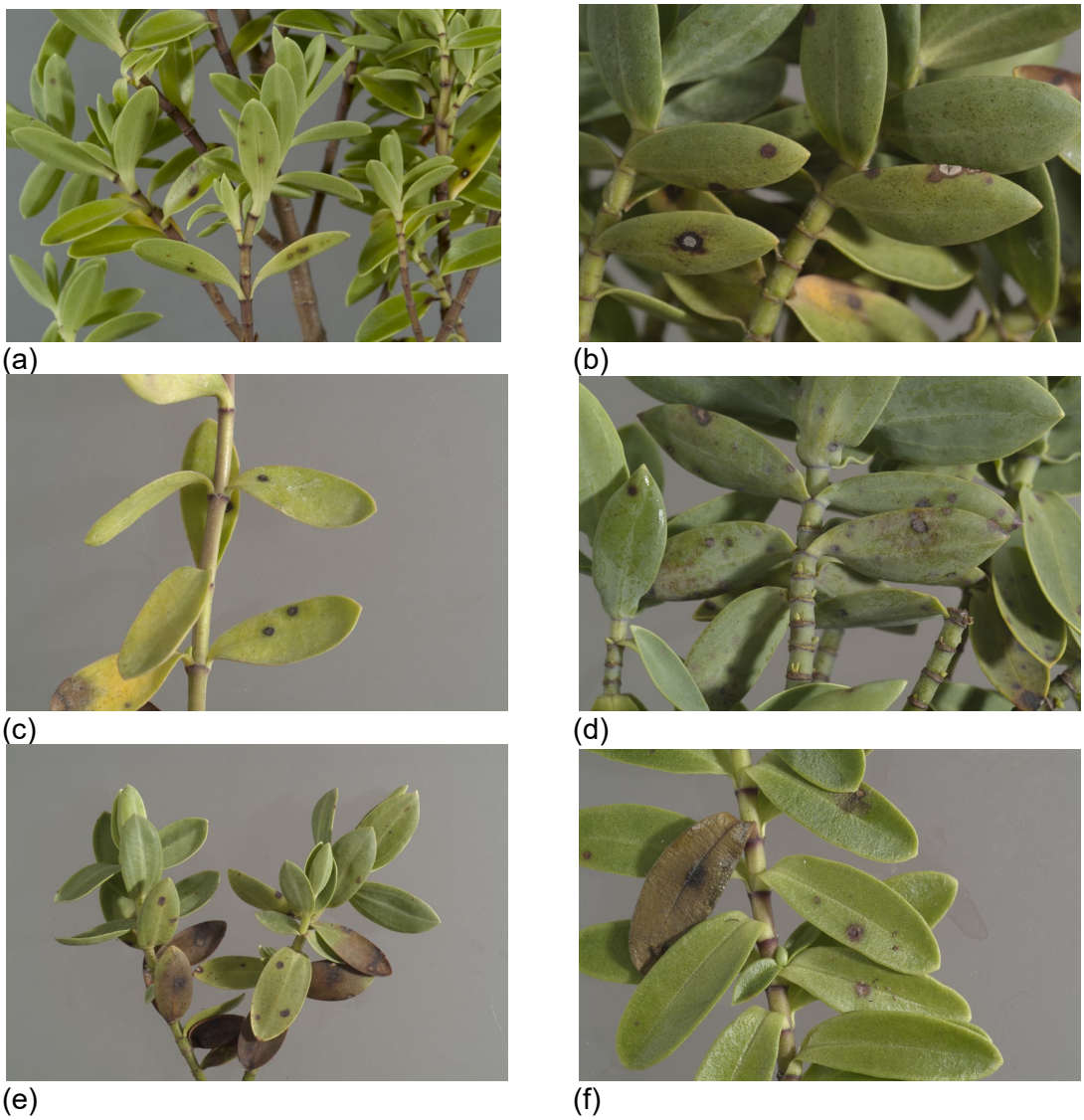
Objective k : Preparation of an Industry Fact sheet on Leaf Diseases of *Hebe*.
A Fact sheet, which will provide information on the various leaf diseases of *Hebe*, including *Stemphylium* leaf spot, will be prepared towards the end of the project in early Spring 2006.

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 6a-f) and most samples yielded a *Stemphylium* sp. None of the samples examined were infected with *Septoria* leaf spot.

plates 6a-f: A range of leaf spot symptoms on samples submitted in the initial grower survey.

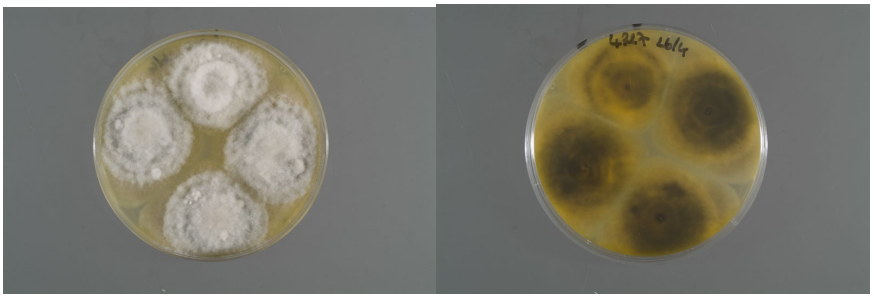


(a) (b) (c) (d) (e) (f)

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, or on the fact that the *Stemphylium* sp. from *Hebe* often produces a characteristic yellow staining of the agar in culture (Plates 7a-b).

plates 7a-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. Of particular significance, no evidence of *Septoria exotica* was detected on any of the samples submitted.

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 8).

plate 8 : Initial symptoms of leaf spot infection following artificial inoculation with *Stemphylium* sp.

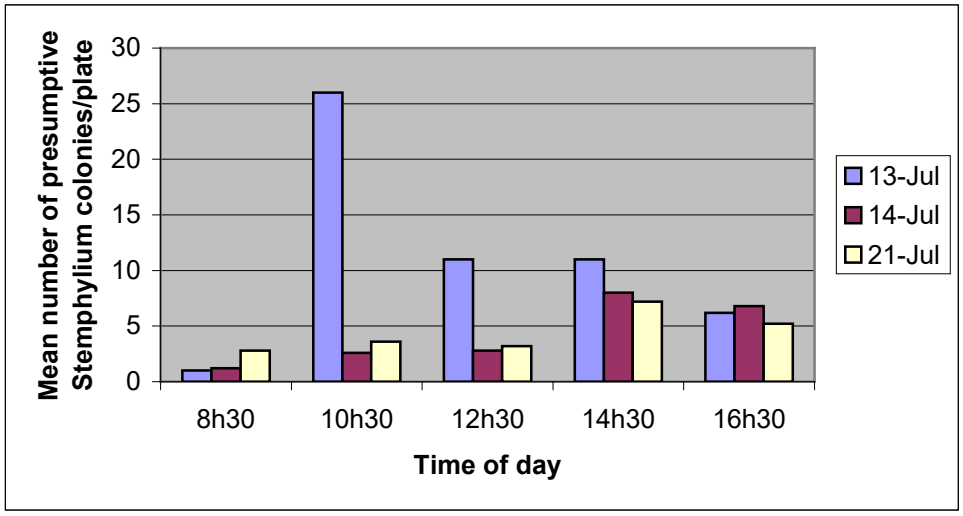
Lesions expanded and developed over the next 7 days on all inoculated leaves (see Plate 9 below). Lesion development was seen both with and without wounding and this suggests that the pathogen is able to enter the host tissue either via natural openings (stomata) or via the production of an infection peg. Necrosis was not observed on the uninoculated control leaves. Early observations 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 9 : Established leaf spot lesions on *Hebe* cv. Red Edge following artificial inoculation with *Stemphylium* sp. 9 days earlier.

Objective c : Examining spore dispersal

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 1.

Figure 1. Incidence of *Stemphylium* spores over an 8hr period on 3 dates



Statistical analysis 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 1) do suggest that generally there were more spores released in the afternoon, than during the morning, which could be due to temperature dependence.

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 infection during the winter months is relatively low. Further testing will be carried out during the spring and summer of 2005 to expand on the collected data.

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

This work has been re-scheduled to be undertaken in April 2005.

Objective e : Investigating the Role of Leaf Litter in Disease Epidemiology

Samples of leaf litter collected from infected sites have been monitored regularly by both CSL and STC by retaining infected leaves under contrasting wet and dry conditions. To date the sexual or perfect stage of the fungus (*Pleospora*) has not been detected on any of the leaf litter. Further samples will be collected in early spring to determine if the *Pleospora* stage can be found following a period of low temperature vernalisation.

Objective f : Studying Varietal Susceptibility to the Disease

This work has been re-scheduled to be undertaken in Spring 2005.

Objective g : *In vitro* fungicide screening

The *in vitro* fungicide screen gave very clear results regarding chemical sensitivity (Figure 2). Perhaps of most significance is the fact that all isolates of *Stemphylium* tested were totally insensitive to carbendazim (Bavistin), which signifies 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 would be required to distinguish the exact explanation. 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 test were iprodione (Rovral), boscalid + pyraclostrobin (Signum), fluazinam (Shirlan) and pyrimethanil (Scala), 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. No clear patterns regarding improved/poorer sensitivity to the chemicals were seen and this suggests that no geographical differentiation is 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 assessment carried out in the experimental, but infected, crop prior to the commencement of fungicide applications indicated moderate amounts of leaf spot, in all the plots, with little variation seen either in the mean number of lesion free leaf pairs/plant (ie 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 4, Table 1a). A second disease assessment was carried out at the mid-point of the experiment and following two fungicide applications. The results of this assessment (Appendix 4, Table 2a) indicate 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 (T1).

A third disease assessment on the 15 November, 11 days after the final (4th) fungicide application. The data from this assessment (Appendix 4, Table 3a) indicates that the growth of the plants remained constant across treatments for the duration of the trial. Therefore, the application of the fungicides do not appear to have had any detrimental (phytotoxic) effect on plant growth at the concentrations and conditions used in this experiment. It also suggests that the pathogen does not affect the rate of plant growth adversely, but instead reduces plant quality severely in the absence of effective fungicide application.

The spread of *Stemphylium* 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 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 are disappointing as the *in vitro* studies suggested they provided an 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 indicate there are a number of potential fungicides that could be used by growers to provide effective control against *Hebe* leaf spot caused by *Stemphylium* sp. The most effective group of fungicides in the trial were the strobilurin fungicides, Signum and Amistar. Although Shirlan, a dinitroaniline fungicide, and Rovral, a dicarboximide, also provided a significant and high level of disease control. Signum provided the best protection of newly emerging leaves, with an increase of only 0.3 new infections per shoot over a period of 62 days, compared to 2.6 in the control treatments.

Finally, a disease assessment in early February 2005, 3 months after the final fungicide application (Appendix 4 Table 4a) suggests that Signum maintained a good 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 this is a particularly encouraging result for the industry, growers are, in the short-term at least, urged to treat the results with some caution. The work has only been conducted on a single susceptible cultivar cv. Red Edge; it is possible that some of the fungicides applied under different conditions to a range of different cultivars could cause some phytotoxicity. Moreover, 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.

Figure 2. *In vitro* fungicide screen – percentage inhibition of mycelial radial growth on agar in the laboratory

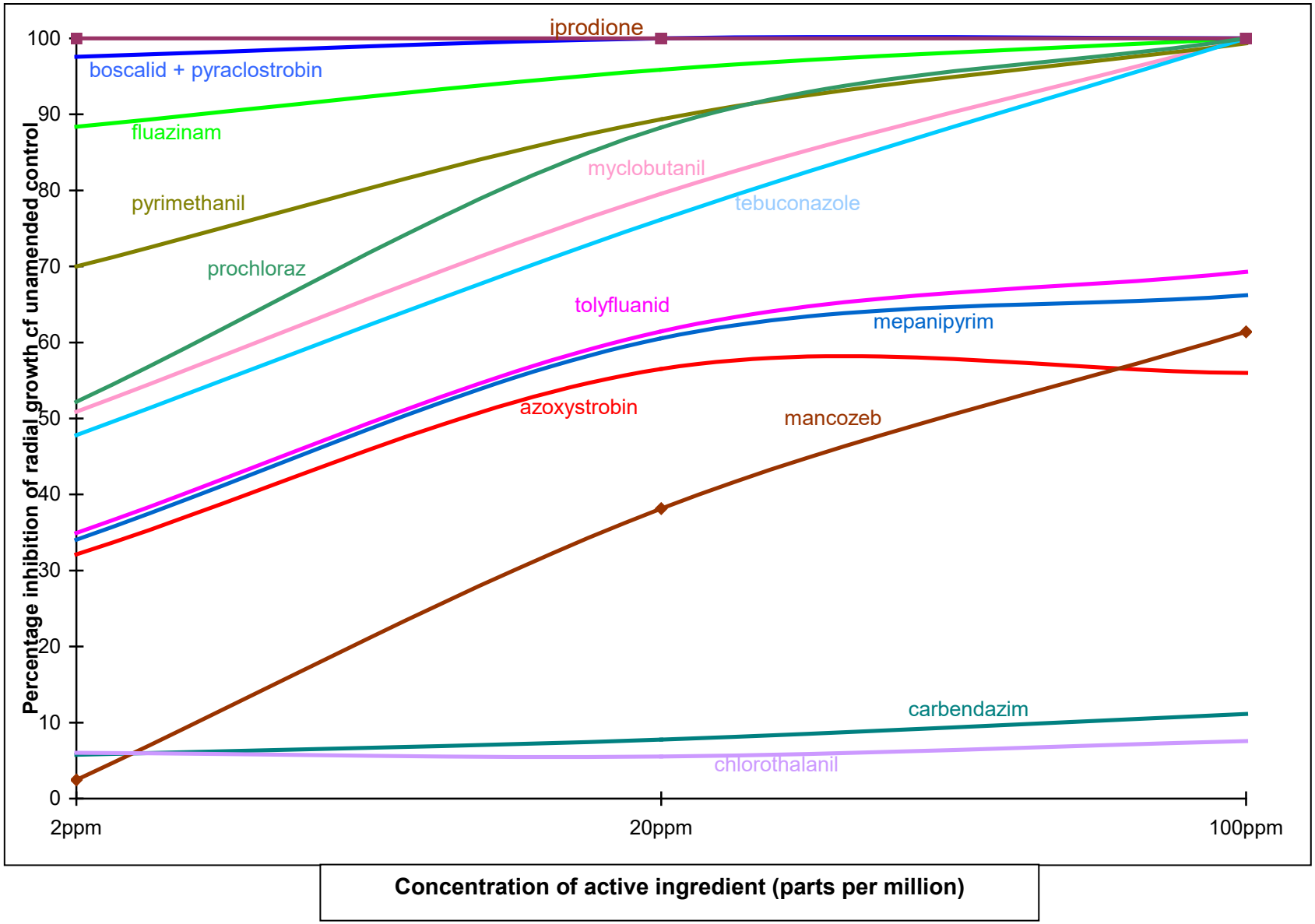
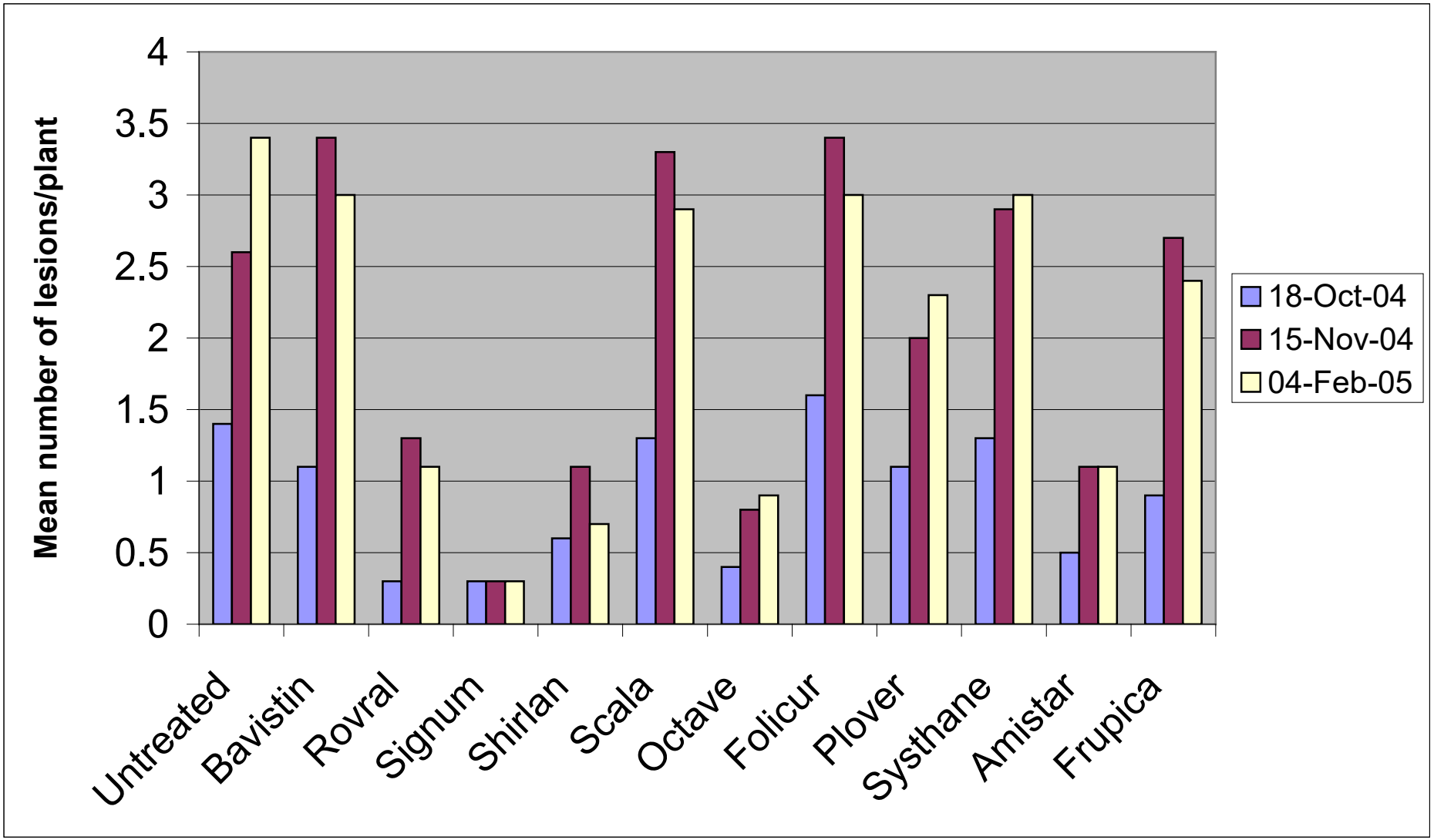


Figure 3 – Fungicide Efficacy Field Trial – Development of *Stemphylium* leaf spot across the treatments over 3 assessment dates.



Objective l : Ensuring Crop Safety with Applied Fungicides

This component of the project is scheduled to be undertaken in late Spring - early Summer 2005.

Objective j : Investigating Fungicide Timing relative to the Infection Cycle

This component of the project is scheduled to be undertaken in late Summer 2005.

Objective k : Preparation of an Industry Fact sheet on Leaf Diseases of *Hebe*.

A Fact sheet, which will provide information on the various leaf diseases of *Hebe*, including *Stemphylium* leaf spot, will be prepared towards the end of the project in early Spring 2006.

Discussion

The project commissioned by HDC on behalf of the industry was prompted following a nursery report of disease control failure in *Hebe*. A severe leaf spot problem in *Hebe* remained uncontrolled and caused significant crop loss following repeated application of the normal fungicide programme used on Hardy Ornamentals. Initial investigations at STC suggested the causal fungus to be a *Stemphylium* species rather than the more usual *Septoria* spp., which were targeted by the usual 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 miss-diagnosed by growers as *Septoria* leaf spot and, accordingly, was being treated with carbendazim routinely.

In the first year of the project we have been 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. It 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 immediately 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 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. Instead, it remains possible that the fungus could have an inherent tolerance to the chemical.

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 and growers can therefore already move forward with confidence and adjust their spray programmes accordingly, subject to ensuring that the various products are approved for use in their specific situation. However, broader crop safety studies on a wider range of cultivars have yet to be undertaken and therefore growers should proceed with caution when using a new fungicide for the first time.

Epidemiological studies have also commenced to investigate the methods of infection, spore survival and dispersal and to identify the optimum conditions for infection and symptom expression. Several of these studies remain ongoing and will be reported later.
However, already initial studies at CSL 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.

So far, work to investigate the mechanisms, timing and influencing factors in spore dispersal have been somewhat inconclusive. The results of the initial spore trapping experiments carried out in June 2004 did not suggest any clear diurnal rhythm to conidial spore release. However, there was some evidence that more spores are released during the early afternoon than at other times of the day, which may be indicative of a temperature response. Later tests carried out in November to investigate the influence of irrigation/rainfall on spore dispersal was considerably hampered by the almost complete failure of the plants to release spores. However, this observation is interesting because it possibly supports the hypothesis that temperature is influential on spore dispersal, and suggests that inoculum levels in the environment are very low during the cold winter months. Further work on spore trapping and the role of ascospores in disease spread will continue in 2005.

In addition to this, further work will commence in the 2nd year of the project and will focus on several key elements of the project:

- To undertake a crop safety screen with short-listed fungicides to investigate the relative safety of ½ N, N and 2N rates of application.
- To investigate the optimum timing of fungicide programmes for maximum economic efficacy.
- To continue to investigate the mechanisms of spore dispersal, and the role of leaf litter in the over-wintering survival of the pathogen.
- To continue to study the optimum conditions for disease spread and infection.
- To determine the relative susceptibility of commercially available cultivars to the disease.
- To prepare a fact sheet for the industry and other stakeholders.

Conclusions

- Leaf spot on *Hebe* species is a widespread and severe problem in the UK.

- Historically, the leaf spot has been attributed to infection by *Septoria exotica*.

- Initial problems reported by a UK grower in 2003 led to preliminary work by STC which suggested a different fungus, tentatively identified as a *Stemphylium* species, might be responsible for the leaf spot.

- Following commencement of the HDC project, diagnosis using traditional laboratory isolation techniques, backed-up by molecular studies, suggest that the pathogen responsible for leaf spotting is *Stemphylium solani* with a 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 pathogen was re-isolated.

- *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 the fungus in agar tests in the laboratory.

- Interestingly, carbendazim (e.g. Bavistin) proved totally ineffective against the *Stemphylium* in *in vitro* tests, perhaps explaining why the same fungicide applied routinely for *Septoria* control failed to give adequate control of leaf spot caused by *Stemphylium*.

- A replicated *in vivo* fungicide efficacy trial undertaken in Autumn 2004 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 didn't 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 useful 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.

- It is recommended that growers with a leaf spot problem secure an accurate identification of the cause by submitting affected leaf material to a commercial diagnostic clinic.

- *Hebe* growers confirmed as having a *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 a range of cultivars. Further consideration should be made to including other effective fungicides in the programme e.g. boscalid+pyraclostrobin (Signum), subject to broader crop safety testing and 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* will be prepared towards the end of the project.

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.

Appendices

Appendix 1 : Results from Grower Survey
Appendix 2 : Tabulated data from in vitro resistance tests
Appendix 3 : Field trial plan for fungicide efficacy trial at STC
Appendix 4 : Tabulated data from fungicide assessments
Appendix 5 : Questionnaire & Information sheet circulated to growers
Appendix 6 : Gantt chart for project schedule

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)	Cuttings from own stock	Overhead	P	Nov/Dec	Stemphylium & Botrytis
PC3323B	Sapphire	N	23.03.04	Moderate	Aliette/Bav/Bravo (Alt)	Cuttings from own stock	Overhead	P	Nov/Dec	Botrytis & bacteria
PC3323C	Silver Dollar	Y	23.03.04	Mild	Aliette/Bav/Bravo (Alt)	Cuttings from own stock	Overhead	P	Nov/Dec	*Stemphylium*
PC3327A	Red Edge	N	24.03.04	Severe	Octave, Aliette	Cuttings from own stock	Overhead	O	3-4yrs	*Stemphylium*
PC3327B	Great Orme	N	24.03.04	Moderate	Octave, Aliette	Cuttings from own stock	Overhead	O	3-4yrs	*Stemphylium*
PC3327C	Marjorie	N	24.03.04	Mild	Octave, Aliette	Cuttings from own stock	Overhead	O	3-4yrs	*Stemphylium*
PC3327D	Pinguifolia pagei	N	24.03.04	Moderate	Octave, Aliette	Cuttings from own stock	Overhead	O	3-4yrs	*Stemphylium*
PC3328A	Albicans	N	24.03.04		Amistar/Aliette/	Cuttings from own stock	Overhead	O	Spring 2003	*Stemphylium*
PC3328B	Mrs Winder	N	24.03.04	Severe (last winter)	Favour/Sportak	Cuttings from own stock	Overhead	O	Spring 2003	Stemph, Botrytis & Cylindrocladium
PC3328C	Youngii	N	24.03.04	Severe (last winter)	applied fortnightly	Cuttings from own stock	Overhead	O	Spring 2003	*Stemphylium*
PC3328D	Rakaiensis	N	24.03.04			Cuttings from own stock	Overhead	O	Spring 2003	Fusarium, Penic & Cladosporium
PC3329A	Rakaiensis	N	25.03.04	Mild	Amistar/Amistar/Aliette	Cuttings from own stock	Overhead	O		Stemph, Cladosporium & Botrytis
PC3329B	Pinguifolia pagei	N	25.03.04	Mild	applied every 7-10 days	Cuttings from own stock	Overhead	O		*Stemphylium*
PC3329C	Autumn Glory	N	25.03.04	Severe		Cuttings from own stock	Overhead	O		*Stemphylium*
PC3329D	Albicans	N	25.03.04	?		Cuttings from own stock	Overhead	O		Botrytis & bacteria
PC3330A	Rakaiensis	N	25.03.04	Mild	Aliette fortnightly	Cuttings from own stock	Overhead	P	6 wks post	*Stemphylium*
PC3330B	Marjorie	N	25.03.04	Mild		Cuttings from own stock	Overhead	P	potting	*Stemphylium*
PC3330C	Red Edge	N	25.03.04	Moderate		Cuttings from own stock	Overhead	P		*Stemphylium*
PC3331A	Red Edge BG1	N	25.03.04	Mild	Amistar/Aliette/	Bought in cutting/young plants	Overhead	O	Sep-03	*Stemphylium*
PC3331B	Red Edge LG1	N	25.03.04	Moderate	Fungex/Bravo/Novak	Bought in as mature plants	Overhead	O	Mar-04	*Stemphylium*
PC3331C	Red Edge LG2	N	25.03.04	Moderate		Bought in as mature plants	Overhead	O	Mar-04	*Stemphylium*
PC3332A	Red Edge	N	25.03.04	Moderate	Fungex & Amistar for small leaf H.	Cuttings from own stock	Overhead	O	August	Stemph,Cladosporium & Fusarium
PC3332B	S. alpina	N	25.03.04	Mild	Aliette/Caramate/Amistar/Favor on	Cuttings from own stock	Overhead	O	August	*Stemphylium*
	Albicans	N	Not sent	Severe	large leaf *Hebe*s.	Cuttings from own stock	Overhead	O	August	Not sent
PC3333A	Rakaiensis	N	25.03.04	Severe	Repulse in winter.	Cuttings from own stock	Overhead	P&O	Aug-02	*Stemphylium*
PC3333B	Red Edge	N	25.03.04	Moderate	Octave at 1st sign	Cuttings from own stock	Overhead	P&O	Aug-03	*Stemphylium*
PC3333C	Albicans	N	25.03.04	Moderate	of symptoms	Cuttings from own stock	Overhead	P&O		*Stemphylium*
PC3334A	Pink Paradise	N	29.03.04	Mild	Octave/Bavistin/Bravo/Aliette	Bought in as mature plants	Overhead	P	Varies	Botrytis & Penicillium
PC3334B	Pink Pixie	N	29.03.04	Mild	Fortnightly rotated sprays	Cuttings from own stock	Overhead	P		*Stemphylium*
PC3334C	Pink Paradise	N	29.03.04	Mild		Cuttings from own stock	Overhead	P		*Stemphylium*
PC3334D	Caledonia	N	29.03.04	Mild		Cuttings from own stock	Overhead	P		*Stemphylium*

PC3337A	Red Edge	N	31.03.04	Mild	Aliette	Bought in as young plants and	Overhead	P		*Stemphylium*
PC3337B	Albicans	N	31.03.04	Mild		Cuttings from own stock	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	Cuttings from own stock	Overhead	P		No identified pathogens
PC3348B	Pagei	N	2.04.04			Cuttings from own stock	Overhead	P		*Stemphylium*
PC3348C	Red Edge	N	2.04.04			Cuttings from own stock	Overhead	P		No identified pathogens
PC3348D	Rakaiensis	N	2.04.04			Cuttings from own stock	Overhead	P		Cladosporium and bacteria
PC3350A	Fran Variegata	Y	05.04.04	Moderate	Compost tea, Aliette, Amistar, Fubol	Cuttings from own stock	Overhead	P	Autumn	Botrytis
PC3350B	Albicans	N	05.04.04	Moderate	Gold, Drench of Octave/Bavistin when	Cuttings from own stock	Overhead	P	last	Botrytis
PC3350C	Silver Dollar	Y	05.04.04	Moderate	spot seen	Cuttings from own stock	Overhead	P	year	*Stemphylium*
PC3355A	Silver Dollar	N	07.04.04	Severe	Stroby, Bravo 500, Octave, Karamate	Cuttings from own stock	Overhead	P	Autumn	No identified pathogens
PC3355B	Red Edge	N	07.04.04	Severe	3-4 weekly programme	Cuttings from own stock	Overhead	P		No identified pathogens
PC3372	Rakaiensis	N	22.04.04	Moderate	Amistar + Repulse throughout season	Cuttings from own stock	Overhead	O	Worse late	Stemphylium
						Cuttings from own stock		O	summer	
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,	Cuttings from own stock	Overhead	P	Mid April	*Stemphylium* and other organisms
PC3393B	Pink Elephant	N	04.05.04	Moderate	Chlorothalonil	Cuttings from own stock	Overhead	P		*Stemphylium*
PC3393C	Just Judy	N	04.05.04	Mild		Cuttings from own stock	Overhead	P		*Stemphylium*
PC3393D	Valentino	N	04.05.04	Severe		Cuttings from own stock	Overhead	P		*Stemphylium*

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 – Trial Plan for *in vivo* Fungicide Efficacy Trial

Evaluation of Fungicides for the Control of *Stemphylium* leaf spot in Hebe

P45 T9	P46 T11	P47 T12	P48 T1
P41 T6	P42 T5	P43 T10	P44 T6
P37 T11	P38 T7	P39 T4	P40 T8
P33 T1	P34 T8	P35 T2	P36 T3
P29 T7	P30 T12	P31 T3	P32 T10
P25 T3	P26 T6	P27 T7	P28 T4
P21 T12	P22 T10	P23 T5	P24 T11
P17 T4	P18 T2	P19 T1	P20 T5
P13 T8	P14 T1	P15 T6	P16 T9
P9 T10	P10 T3	P11 T9	P12 T2
P5 T2	P6 T4	P7 T8	P8 T7
P1 T5	P2 T9	P3 T11	P4 T12

T1: Untreated
T2: Bavistin
T3: Rovral
T4: Signum
T5: Shirlan
T6: Scala
T7: Octave
T8: Folicur
T9: Plover
T10: Systhane
T11: Amistar
T12: Frupica

Appendix 4 – 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 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

Figure 1. Gantt chart for proposed study on *Hebe* Leaf spot (*Stemphylium* sp.)