PROTEACEAE
Managing Elsinoe scab

A report for the Rural Industries Research and Development Corporation
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June 2000

RIRDC Publication No. 00/64
RIRDC Project No DAV-116A
Foreword

Elsinoe scab of South African Proteaceae is caused by *Elsinoe* spp. The disease causes scabby lesions on stems, leaves and flower heads and heavy infection can cause twisting or splitting of stems. Pruning of affected plants for disease control and/or the disease itself can reduce production and in some cases kill the host plants. The scabby lesions can render the cut flower product unmarketable or marketable only at a reduced price.

A 1994/5 study on the incidence and economic impact of Elsinoe scab (Pascoe, Ziehrl and Porter 1995) was funded by RIRDC and the Australian Flora and Protea Growers Association (AFPGA) and conducted by researchers at the Institute for Horticultural Development. This study found that Elsinoe scab was rated by survey respondents from both plantations and nurseries as the most important disease of cut flower Proteaceae. Loss of production, crop re-establishment and disease control, were determined to be costing the industry in excess of $600,000 per annum. The study also showed that the disease occurred on approximately 27% of plantations in Australia, comprising at least 31% by area of South African Proteaceae production. Existing management strategies for the disease were of limited effectiveness.

Results of this earlier study prompted RIRDC to commission a further research project under the Wildflowers and Native Plants Program in 1995. Three fungicides were identified in this Study which reduce stem disease. All three are non-systemic protectants and provide best control if applied before infection occurs.

This project was funded from RIRDC Core Funds which are provided by the Federal Government and is an addition to RIRDC’s diverse range of over 450 research publications. It forms part of our Wildflowers and Native Plants R&D program, which aims to improve the profitability, productivity and sustainability of the Australian wildflower and native plant industry.

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- downloads at www.rirdc.gov.au/reports/Index.htm
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Acknowledgments and Collaboration

This study was supported with funding from the Rural Industries Research and Development Corporation, the Australian Flora and Protea Growers Association (AFPGA), formerly the Australian Protea Growers Association, and Agriculture Victoria, Department of Natural Resources and Environment.

We thank the AFPGA State and National committee members for their guidance and cooperation with this research and specifically Barry Pontifex, Martin Sayers, Beverley Karpinski and Dennis Morgan. We also thank growers for their willingness to share information, providing specimen material for research, supplying at-cost plants and making available use of land and commercial crop plants for trials.

Appreciation is extended to Denis Tricks and John Cane, Brian Harris, Peter Sypkes (Ausflora Pacific), David and Andrew Mathews and Paul Armitage (Proteaflora) for their valuable advice on research direction and practicalities.

We also thank the following staff at the Institute for Horticultural Development for their assistance at various stages in the project: Cassie Hall, Alastair Crellin, Catriona Moors, Paula Nicholson, James Miller, Norm Morrison, Richard Mapson, Joe Rae, Daniel Isgro, Trish McGee, Andrea Van Der Linden, Alan Shanks, Carolyn Donald, Trevor Davy, Dave Bardon, Peter Cole, Sue Feruglio, Vannessa Hood and Janice Truett, as well as work experience students from schools and universities who have provided technical assistance.

We acknowledge the benefits gained from collaborative work with Professor Pedro W. Crous, Department of Plant Pathology, University of Stellenbosch and Dr Stephen Ferreira and Mr Norman Nagata, Maui Agricultural Research Center, University of Hawaii. We also thank other scientists for imparting their knowledge including Dr Lois Turnbull, Dr John Curtis, Bill Washington, Dr John Faragher, Ann Cass, Wayne Tregea and Tony Slater.

Dr Graham Hepworth deserves a particular thank you for his extensive involvement in the design and analysis of trial work and editing the reporting of this data.
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Executive Summary

Elsinoe scab of South African Proteaceae is caused by Elsinoe spp. The disease causes scabby lesions on stems, leaves and flower heads and heavy infection can cause twisting or splitting of stems. Symptoms vary from species to species and among cultivars and may be difficult to identify particularly during early stages of infection.

A 1994/5 study on the incidence and economic impact of Elsinoe scab determined that the disease occurred in approximately 27% of Protea plantations in Australia and was costing the industry in excess of $600,000 per annum (Pascoe, Ziehrl and Porter 1995). It also showed that fungicide strategies employed by growers were of limited effectiveness.

As of February 2000 there were no chemicals registered for control of Elsinoe scab in Australia. Growers have been i) applying fungicides registered for various ornamental crops, ii) using fungicides off-label, or iii) not applying anything at all. Limited knowledge of the disease cycle resulted in excessive or inadequate fungicide application and generally poor disease control.

Results of the 1994/5 study prompted RIRDC to commission a further research project under the Wildflowers and Native Plants Program in 1995. This project, reported here, was aimed at developing an integrated control program for Elsinoe scab of cut flower Proteaceae with the following objectives:

1. To limit the spread of disease from nursery stock and infested sites to non-infested sites
2. To improve efficacy of control strategies and in particular, to educate growers on effective application methods and timing of fungicides and to obtain data to support registration of fungicides for use on Proteaceae
3. To gain a better understanding of the biology and epidemiology of the disease to assist in better prediction of disease outbreaks and assist in correct timing of chemical sprays
4. To determine key factors associated with infection and disease development
5. To develop an integrated control program for Elsinoe scab of cut flower Proteaceae which:
   - reduces loss of revenue to disease
   - reduces the cost of control and re-establishment
   - reduces chemical usage
   - limits the spread of the disease throughout the industry
   - reduces the threat to development of export markets.

A number of factors were identified in this study as contributing to the economic loss caused by Elsinoe scab. The following management procedures would help reduce loss:

- correct disease diagnosis
- use of reputable plant suppliers
- quarantining of new stock for up to six months
- avoiding the use of overhead irrigation
- taking cuttings for propagation from disease-free plants
- sterilising pruning and harvesting tools prior to use
- pruning out and destroying diseased plant material, particularly small non-productive shoots
- ensuring adequate spray coverage
- applying fungicides when conditions are favourable for disease and before symptoms appear
- testing plants for phytotoxicity before applying new fungicides
- applying fungicides in accordance with label recommendations
- avoiding overcrowding of plants in nursery and field
- selection of resistant varieties
Research outcomes

Briefly, research conducted on Elsinoe scab found that:

- Australian isolates of *Elsinoe* spp. collected from *Serruria*, *Leucadendron* and *Leucospermum* represent the same species as that occurring on *Leucospermum* and *Leucadendron* in South Africa (Swart *et al.* in press).

- Some cultivars and selections of Proteaceae are more susceptible to infection than others and the effect of the disease on plant health and production can vary widely, even with the same level of initial infection.

- Plants are more susceptible to the disease during the early stages of spring and autumn growth flushes.

- Six of eleven retail nurseries surveyed had plants with visible symptoms of the disease and a further 17% of plants without symptoms developed symptoms later. This increased the number of nurseries with disease to seven.

- Within nurseries disease levels may be exacerbated by, overcrowding of plants, overhead irrigation and high humidity. These conditions favour infection and disease progression, particularly on soft new shoots.

- *Elsinoe* spores may be present throughout most of the year on properties with high disease levels.

- Rain or overhead irrigation is required for at least primary infections to occur and moist warm conditions favour disease progression.

- Spore germination on trial plants was significantly reduced at temperatures below 20°C, although some germination did occur on soft new growth and under the pressure of high spore levels.

- The fungus isolated has the ability to infect other genera including *Banksia* and *Dryandra* spp.

- Three fungicides, viz. Octave®, Captan® and Dithane®, reduced stem disease on flower shoots by 75% and significantly reduced the number of severely affected stems per plant.

The three most effective fungicides identified in this study were all non-systemic protectants and will therefore provide the best control if applied before infection occurs. It is particularly important to apply fungicide sprays to protect young spring and autumn growth.

This study has also identified that Proteaceae hosts can carry *Elsinoe* without showing symptoms. Development of a nursery hygiene program may help reduce spread of the disease, and a suitable program should be based on training on how to minimise conditions that promote *Elsinoe* spread and survival.

Finally, since some Australian native Proteaceae were able to be infected with *Elsinoe* spp. isolated from South African varieties, and therefore have the ability to harbour the disease, a broad review of plant hygiene and distribution practices within the Australian Protea and Wildflower industries may be warranted.
1. Introduction

Elsinoe scab of South African Proteaceae is caused by *Elsinoe* spp. The disease causes scabby lesions on stems, leaves and flower heads and heavy infection can cause twisting or splitting of stems. Pruning of affected plants for disease control and/or effects of the disease itself can reduce production and in some cases kill the plants. The scabby lesions can render the cut flower product unmarketable or marketable only at a reduced price.

The disease was first recorded in South Africa by Benic and Knox-Davies (1984) and in Victoria, Australia in 1985 (Victorian Department of Agriculture, Plant Disease Herbarium data). It has since been found in all eastern states of Australia and unconfirmed reports have been received from Western Australia.

Elsinoe scab of Proteaceae has now been confirmed on *Protea* species in South Africa, Zimbabwe, California, Hawaii and Australia (Swart et al. in press). Several species of *Elsinoe* occurring on Proteaceae have recently been described by Swart et al. who compared Australian isolates to South African and other *Elsinoe* isolates. Australian isolates collected from *Leucadendron*, *Leucospermum* and *Serruria* represent *Elsinoe leucospermi* L. Swart & P. Crous, the same species as that occurring on *Leucospermum* and *Leucadendron* hosts in South Africa (Swart et al. in press). An Australian isolate from *Banksia* was described as a separate species, *Elsinoe banksiae* I. Pascoe & P. Crous.

Symptoms of Elsinoe scab vary between species and cultivars and may be difficult to recognise particularly at early stages of infection. This report shows that *Elsinoe* isolated from South African varieties has the ability to infect at least some native genera including *Banksia* and *Dryandra*. In terms of the Wildflower and Protea industries in Australia, disease on these plants may assist the spread of *Elsinoe* or become a matter for future economic concern.

There are no chemicals currently registered specifically for control of Elsinoe scab in Australia. Growers therefore use fungicides broadly registered for ornamentals, apply chemicals off-label or do not spray at all. Results presented in this report will enable growers to better understand the disease and thus target pruning and spray application more effectively. This will result in better production and marketability of affected crops, savings on disease control costs and reduced levels of disease both on properties and as a source for spread of the disease.

Potential or continued problems for the industry are seen as:
- continued spread of the disease due to lack of early detection by growers, latency of symptom expression or inadequate hygiene/quarantine practices
- harbouring of *Elsinoe* by non-suspect varieties and selections, including some natives
- increased prevalence of disease on high value crops such as *Protea*
- potential *Elsinoe* infection of commercially grown natives such as *Dryandra formosa*

Elsinoe scab of Proteaceae may be a problem for both the Wildflower and Protea industries, rather than the latter alone. Joint strategies for hygiene, plant distribution and accreditation of retail nurseries should be developed.

The research proposal for this project was formulated with RIRDC and industry members to identify timely and cost-effective control strategies. The research relied heavily on field trials to allow direct application of outcomes to commercial properties. Extended and unusual weather conditions over nearly the entire period of one trial however, and high spore levels, impeded the collection of important data on conditions conducive to infection and disease development. This was seen as a major drawback in understanding the disease and for the development of further trials. In 1998, RIRDC funded a nine-month extension to the project and researchers undertook to identify key epidemiological factors through laboratory work. The outcomes of this work are also reported here.

1.1 Characteristics of *Elsinoe* sp. of Proteaceae
The causal fungus of Elsinoe scab, which occurs on *Leucadendron*, *Leucospermum* and *Serruria* in Australia has recently been described as *Elsinoe leucospermi* L. Swart & P. Crous (Swart *et al.* in press) and confirmed as the same species infecting *Leucospermum* and *Leucadendron* spp. in South Africa.

Symptoms of Elsinoe scab vary between species and cultivars and early stages of infection may be particularly difficult to identify. Typical symptoms are the development of reddish raised lesions on stems and leaves. These become scab-like and develop a pale corky appearance as they progress (Ziehrl, Pascoe and Porter 1996). Variations to these symptoms are common, however. For example, inoculation of *Leucospermum cordifolium* in a glasshouse trial, resulted in the development of numerous dark sunken spots, which later progressed to form more typical *Elsinoe* lesions. Infection of a *Banksia* sp. with isolates from South African plant varieties, resulted in round to irregular grey lesions with a brown margin on leaves, and *Dryandra formosa* developed a general and irregular corky pattern on stems. Disease symptoms have been observed as early as seven days after inoculation.

Ziehrl, Pascoe and Porter (1996) described lesions as occurring in clusters on stems and leaves, due to specific events that occurred during shoot extension and under conditions favourable for infection. More recently it has been discovered that infection may occur throughout the year if conditions are appropriate (refer section 6), but the disease most commonly occurs on the soft new material of extending shoots.

Heavy to moderate infection of sensitive varieties such as *Serruria florida*, may cause abnormal growth and twisting of the stems and older lesions may cause localised splitting of the stem. Some varieties and selections are more susceptible to infection than others and varying degrees of post-infection lesion development and plant damage have been observed. *Serruria* spp. for example, with similar levels of disease to *Leucadendron* plants may die in the absence of adequate disease control measures, while *Leucadendron* plants may continue to produce marketable stems with little or no disease control. Even so, diseased plants are less productive and stem marketability is reduced.

*Elsinoe* is an Ascomycete fungus that produces two types of spores, conidia and ascospores. The former are produced asexually (*Sphaceloma* anamorph) and have been the only type of spores observed for *Elsinoe* in Australia (Pascoe, Ziehrl & Porter 1995). They are small slimy spores dispersed primarily by rain or irrigation water splash. Wind may assist the travel of these water droplets and therefore also the spores.

Spread of the disease within and between properties can occur through the use of unsterilised harvesting and pruning tools, use of infected plants for cuttings and introduction of diseased planting stock. Within nurseries, disease levels may be exacerbated by the close proximity of plants, mist/bench irrigation and high humidity. These conditions tend to encourage both the development of soft vulnerable new plant growth and infection and disease progression.
1.2 Previous studies in Australia

A 1994/5 survey on incidence and economic impact (Pascoe, Porter and Ziehrl 1995) showed that the disease has spread rapidly and threatens domestic and export markets. Open trade of plants from affected nurseries and plantations, difficulties in disease identification and ineffective control, are seen as the primary reasons for spread of the disease throughout the eastern coast of Australia. The survey found that only 20% of growers who pruned out infected material believed this was effective and only 19% believed that chemical regimes provided good control of the disease.

Other survey findings included:

- Elsinoe scab was rated by survey respondents from both plantations and nurseries as the most important disease of cut flower Proteaceae.
- Loss of production, crop re-establishment and disease control cost the industry in excess of $600,000 per annum
- Elsinoe scab occurred on approximately 27% of plantations in Australia, comprising at least 31% by area of South African Proteaceae production
- Fungicide and management strategies employed by growers were of limited effectiveness

2. Objectives

The objectives of the research project were:

1. To limit the spread of disease from nursery stock and infested sites to non-infested sites
2. To improve the efficacy of control strategies and in particular, to educate growers on effective application methods and timing of fungicides and to obtain data to support registration of fungicides for use on Proteaceae
3. To gain a better understanding of the biology and epidemiology of the disease to assist in better prediction of disease outbreaks and assist in correct timing of chemical sprays
4. To determine key factors associated with infection and disease development
5. To develop an integrated control program for Elsinoe scab of cut flower Proteaceae which;
   - reduces loss of revenue to disease
   - reduces the costs of control and re-establishment
   - reduces chemical usage
   - limits the spread of the disease throughout the industry
   - reduces the threat to development of export markets
3. **Achievement of Objectives**

This report details problems and practices associated with control of the disease, new research methods employed in conducting trials, susceptibility to the disease of a number of South African and Australian native varieties and selections and efficacy of fungicides. It also includes the outcomes of research on the biology and epidemiology of the disease.

The field, glasshouse and laboratory trials conducted to achieve the objectives of this project are summarised below, together with some of the primary outcomes.

**Objective 1. To limit the spread of disease from nursery stock and infested sites to non-infested sites**

- A survey of retail nurseries and investigation of latency of disease identified Elsinoe scab symptoms and latent disease on South African Proteaceae in Victorian retail nurseries. A recommendation is made in this report to introduce a nursery hygiene program to help reduce spread of the disease.
- A glasshouse trial to investigate the susceptibility of South African and Australian native *Proteaceae* identified cross pathogenicity of *Elsinoe* species. The ability of an *Elsinoe* isolate from the native Banksia affect South African Proteaceae, and of isolates from South African varieties to infect a number of Australian native Proteaceae, highlights the potential that these plants may also contribute to spread of the disease. It was recommended at the 5th Australian Wildflower Conference (Melbourne 1999) and in this report, that a broad review be undertaken of current hygiene and distribution practices within the Australian Protea and Wildflower industries.

**Objective 2. To improve efficacy of control strategies and in particular, to educate growers on effective application methods and timing of fungicides and to obtain data to support registration of fungicides for use on Proteaceae**

- Results from a field trial in Victoria showed that *Elsinoe* spores may be present throughout most of the year on a property with a high level of disease. Data indicated that a threshold of 20°C may exist, below which the potential for spore infection is greatly reduced.
- Results from a fungicide trial in Victoria showed that:
  - effective control of Elsinoe scab must be based on good management and hygiene practices, including removal of diseased material and, in particular, small non-productive shoots that remain after harvest.
  - efficacy of chemical disease control is dependent not only on the product used, but also on spray application techniques that will provide even coverage of crops and individual plants.
  - Octave®, Captan® and Dithane® achieved a 75% reduction of disease on the surface area of marketable *Leucadendron* stems. In this trial, diseased material was not removed prior to fungicide application and minimal attention was given to hygiene measures. It is therefore anticipated that a much greater level of disease control can still be achieved. These fungicides also significantly reduced the number of severely affected stems. All chemical treatments applied achieved some level of control on marketable (flower) shoots of *Leucadendron* plants.
  - Data showed that fungicides providing the greatest control were non-systemic and suggested these not only protect plant material from infection, but also hinder disease progression. Data from this trial can be used to support future applications for registration of fungicides, for the control of Elsinoe scab.
- Information gathered throughout these trials was reported in Buds and Bracts, RIRDC milestone reports, AFPGA annual conferences and the 5th Australian Wildflower Conference (Melbourne 1999) and is also detailed in this publication.

**Objective 3. To gain a better understanding of the biology and epidemiology of the disease to assist in better prediction of disease outbreaks and assist in correct timing of chemical sprays**
Collaborative work on the characteristics of *Elsinoe* showed that the *Elsinoe* species found on *Leucospermum*, *Leucadendron* and *Serruria* plants in Australia is the same as that occurring on *Leucadendron* and *Leucospermum* in South Africa (Swart et al. in press).

Laboratory studies found that spore formation can be induced by water irrigation of lesions and subsequent moist incubation at 22°C. It was also shown that infection and disease development can be promoted by placing plants inside a bench-top polyhouse with regular misting (under plastic) for 48 hours. This increased both the temperature and humidity levels during this period.

Results from a field trial conducted to identify environmental conditions that favour spore release and infection, showed that *Elsinoe* spores may be present throughout most of the year on a property with high disease levels. Data indicated that a threshold of 20°C exists, below which the potential for spore infection is greatly reduced. However, the plant growth stage appears to be a critical factor for primary infection to occur and very young growth is at risk from infection even at temperatures below 20°C when spore levels are high. Early plant growth should therefore be protected against infection.

Results from these trials have been reported at conferences and in industry newsletters, *Buds and Bracts*, RIRDC milestone and compendiums and IHD annual reports.

**Objective 4. To determine key factors associated with infection and disease development**

Field results showed that infection of bait plants occurred even where the total rainfall was as low as 6.0 mm in one sampling fortnight.

An investigation of latency of disease (as part of a retail nursery survey) found that 17% of symptom-free plants collected could be induced to express *Elsinoe* scab. The time between infection and disease development is thought to be largely dependent on appropriate humidity and temperature conditions and this may occur immediately following infection or be delayed.

A glasshouse trial conducted to investigate the susceptibility of South African and Australian native Proteaceae identified cross pathogenicity of *Elsinoe* species; the ability of an *Elsinoe* isolate from the native Banksia to infect South African Proteaceae plants and of *Elsinoe* isolates from South African varieties to infect a number of Australian native Proteaceae.

Information from these trials has been reported at conferences and in industry newsletters, *Buds and Bracts*, RIRDC milestone and compendiums and IHD annual reports.

**Objective 5. To develop an integrated control program for Elsinoe scab of cut flower Proteaceae**

Research results have been disseminated throughout the project period by direct contact with growers and grower groups, newsletters, conference papers and industry articles. This report however, is the first comprehensive account of project outcomes and implications for both the Protea and Wildflower industries.

An integrated approach to the control of *Elsinoe* scab is detailed in the “recommendations” of this report. Together with “further action and dissemination of outcomes” in the same section, this addresses all the key issues listed above.

The field trials conducted were an important component of industry-based research. However, current pressure to conduct extensive and ‘practical’ field research, should not replace the acquisition of basic information that can only be gained through laboratory and glasshouse trials. This information may be critical for the practical design of field trials and application of control methods. It is recommended that in the conception of new research projects, all parties carefully consider the importance of basic studies that may be required to fulfil project objectives.
4. Laboratory Studies on Sporulation of *Elsinoe* and Infection and Disease Development Processes

To successfully conduct field and glasshouse trials aimed at developing an IPM strategy for Elsinoe scab, it was first necessary to evaluate experimental procedures used in the study of similar diseases and to develop new protocols for work with Elsinoe scab. Several procedures developed as part of this project are outlined below and were used in this study to induce infection and disease development on trial plants and for the assessment and disease diagnosis of trial and sample plant material.

4.1 Promotion of disease development by a bench-top polyhouse system

A bench-top polyhouse system was designed to promote infection and disease development processes, by increased temperature and humidity conditions during incubation. This system was modelled on one used by Williamson and McNicol (1989) who, working with *E. veneta* (Burkh.) Jenkins, covered inoculation sites of raspberry cultivars with polyethylene bags, to maintain high humidity during incubation. This system was used to:

1. Promote disease development on Proteaceae bait plants used to measure spore dispersal and infection at field sites affected by Elsinoe scab
2. Promote disease development on symptom free plants collected from retail nurseries and therefore determine if the expression of disease may be latent or suppressed
3. Encourage infection and disease development on glasshouse plants inoculated both with spores and with crushed *Elsinoe* culture.

The polyhouse was constructed from PVC plumbing pipes and covered with heavy duty clear plastic. It measured 1200mm x 970mm x 550mm, with an extra 550mm at the apex. Round top polyhouses were also used. A single misting nozzle was mounted on the centre pipe, with an output volume of 20L per hour. This was programmed to run three times daily for three minutes each time. Plants were placed on a tin tray that held approximately one centimeter of water before overflow. This provided the water for increased humidity and misting ensured that the leaf material was kept moist.

Inoculated plants were placed into the polyhouse system approximately 30 minutes post inoculation. All plants were held in the polyhouse for a period of 48 hours, but the plastic was loosened after 24 hours to allow some airflow. Average relative humidity values for the polyhouses ranged from 74% to 90%. Minimum and maximum temperatures recorded were 17.0 °C and 41.5 °C respectively with an average of approximately 25 °C.

Plants collected from field or nursery sites were placed apart to avoid transfer of fungal hyphae or spores through contact of shoot material. A preliminary trial was conducted to test whether misting could assist transfer of spores from inoculated plants to un-inoculated plants and infect the latter. Results from this trial were negative and the system proved very successful.
4.2 Conidial spore production from field collected shoots

An experiment was conducted to determine if Elsinoe lesions on field collected Proteaceae stems could be induced to sporulate.

Diseased Leucospermum and Leucadendron shoots were field collected in Victoria. These were refrigerated 4°C or less within an hour of collection and used within 12 hours. For each species, three replicates were used for the following treatments:

1. Shoot lesions were flushed with a strong flow of tap water for half an hour, rinsed in sterile distilled water (SDW) and moist incubated in the dark at 22°C for 24 hours.

2. Lesions were irrigated with medium flow tap water for 24 hours, rinsed in SDW and moist incubated in dark at 22°C for 24 hours.

3. Shoot lesions were flushed with medium flow tap water for half an hour, rinsed in SDW and a) kept wet in SDW for three hours or b) kept moist only with SDW. All shoots were then incubated at 22°C in the dark for 24 hours.

For all treatments, the plant material and lesions in particular became very soft. Sectioning and microscopy results revealed:

- the presence of spore masses on all diseased Leucospermum stem areas
- treatment three (above) applied to Leucospermum plant material, provided the largest percentage of spore masses for all treatment/plant combinations
- spore development on Leucadendron stems occurred only with treatment one
- the only recorded spore mass on leaf material for this experiment occurred on Leucospermum material with treatment two.

These outcomes enabled production of inoculum for use in glasshouse experiments. The third treatment was employed successfully on a number of occasions during the study. However, a laboratory trial initiated in September 1999 was eventually aborted when diseased field and glasshouse collected material failed to sporulate using any of these treatments. Prior to this trial, material was collected from December to March. It is possible that unknown environmental or intrinsic factor/s may inhibit sporulation at specific times of the year, even when plant material is treated under controlled conditions following collection.

4.3 Spore harvest and inoculation

Spores were collected by immersing stem lesion areas in a 200ml solution of 0.02 Tween 80 and distilled water and brushing lightly with a camel hair brush to promote release of spores. This was based on the technique employed by Williamson, Hof and McNicol (1989) to release E. veneta conidia from media cultures. Eighteen stem sections approximately 15 cm in length, yielded a spore concentration of around 8 x 10^5 spores/ml.

A fine spray of this solution was applied directly to young shoot material of potted plants, similar to the method used by Whiteside (1975). Following treatment in the bench-top polyhouse system plants were placed under semi-controlled glasshouse conditions (generally 15-25°C, > 50% RH and watering three times daily for 3-5 minutes at a time). Lesions were observed as early as seven days post-inoculation and a high rate of infection was achieved on Leucospermum, Leucadendron and Serruria plants.

Inoculation using culture isolates was used for a varietal susceptibility trial (Section 7). The method involved blending cultures in distilled water for 30 seconds and spraying this solution onto young shoot material.
4.4 Disease assessment chart for *Leucadendron*

A rating system was developed to assess the average percentage of leaf or stem area diseased with Elsinoe scab and to obtain information on the distribution of the lesions over this area (Figure 4.1). In combination with area diseased, the distribution of lesions can provide some understanding of whether many spores caused initial infection, which may or may not have progressed further, or if lesions developed from only a small number of infection points.

![Figure 4.1 Rating system for assessment of disease severity and distribution on *Leucadendron* stems and leaves](image)
4.5 Laboratory trial 1999

Introduction
In order to establish a prediction model that allows precise determination of both infection and disease development, it is necessary to clearly define environmental and biological factors that promote sporulation, spore germination and infection of new shoot material, and progression of lesions on affected shoots. Field and laboratory trial results detailed in this report, together with available literature, have shown that infection of new plant material is influenced by moisture levels, temperature, spore pressure due to on-property disease and the plant growth stage (refer figure 9.1). Increased temperature and humidity conditions were also shown to promote disease development from infection sites (refer section 4.1). Figure 9.1 serves as a guide to potential infection periods, but to further delimit a prediction model, information is still required on:

1. the amount and duration of free water required for spores to be transferred and undergo germination on new plant material, for both primary and secondary infections
2. the range of temperature and humidity conditions that allow infection and disease progression
3. the susceptibility of the host at different growth stages
4. how all these factors interact

This trial attempted to define the temperature and humidity conditions of spore germination and disease progression (point 2).

Objectives
To determine key temperature and humidity values associated with;
1. spore germination and infection
2. disease development

Methodology
Trial design
Spore germination was to be tested on individual leaves of *Leucospermum* plants and assessed as present or absent. The trial was established using twelve treatments; four relative humidity levels by three temperature levels, with four replicates per treatment and two leaves per replicate.

Design of the disease progression investigation was based on spore germination results. If spore germination was high within a narrow range of humidity and temperature, the same leaves could be assessed for disease progression. However, if spore germination was low and/or over a wide range of conditions, a new trial with a higher number of replicates would need to be undertaken to measure the level of progression over time.

Temperature and humidity conditions
Desired relative humidity levels at specific temperatures were attained with glycerol and water solutions using the method described Braun & Braun (1958) and Forney and Brandl (1992). Temperature and humidity values used were 10°C, 20°C and 30°C and 40%, 50%, 70% and 90% respectively.

Experimental procedure
Glass vessels with the appropriate glycerol/water solutions (Braun & Braun 1958 and Forney and Brandl 1992) were placed in temperature-controlled units at 10°C, 20°C and 30°C and covered with vaseline-sealed glass lids to allow conditions to equalise.
Leucospermum shoots with Elsinoe lesions were collected from a Victorian field site and a glasshouse at IHD and treated for conidial spore production (as detailed in section 4.2). These conidial spores were required for spore germination and disease progression investigations of this experiment.

Results and Discussion
Sporulation failed to occur on all collected material. This procedure to obtain spores was repeated several times using different plant varieties and varied source locations. The trial was finally aborted in November 1999 after sporulation failed to occur under a variety of conditions. Interestingly this procedure had been used numerous times throughout the project and had always met with success. It was noted however, that previous material had been collected in the months of December to March. It is possible that unknown environmental or intrinsic factor/s inhibit sporulation at specific times of the year, even when plant material is treated under controlled conditions after collection.
5. Survey of Retail Nurseries and Investigation of Latency of Disease

Introduction
The trade of *Elsinoe* affected plants is seen as one of the primary reasons for the spread of the disease throughout eastern Australia. In 1994/5, as part of the industry survey conducted by IHD, it was found that many plantation and nursery growers were still unaware of Elsinoe scab or unable to identify symptoms of the disease. Growers however, also reported development of Elsinoe scab on plants that were purchased with no visible disease symptoms. This prompted research to determine i) the level of disease in retail nurseries and ii) whether latency of disease was occurring and consequently contributing to the spread of Elsinoe scab.

Objectives
A survey of retail nurseries was conducted in 1997 to determine whether:

1. nurseries stocked Proteaceae with *Elsinoe* symptoms and
2. plants lacking visible disease symptoms could be induced to develop disease, ie. to determine whether infection may have occurred at some stage prior to sale, without further progression of the disease or expression of symptoms.

Methodology
Selection of nurseries
The Yellow Pages ® (Telstra Corporation) 1997 Australian listing were consulted to identify retail nurseries within the Melbourne metropolitan area. Twelve nurseries were then selected on the basis of stocking South African Proteaceae.

Disease survey technique
The twelve targeted nurseries were visited from November 1997 to December 1998. All plants from genera known to have the potential of carrying the disease were inspected for visible *Elsinoe* lesions.

Selection of plants for latency experiments
Six of the retail nurseries investigated stocked at least four South African Proteaceae plants with no visible *Elsinoe* symptoms and were therefore suitable for investigating latency of disease development.

Survey, treatment and assessment of latency of disease development
Four symptom-free plants were bought from each of the six nurseries mentioned above and subjected to polyhouse conditions (refer section 4.1), to promote disease development on plants with possible (but not visible) existing *Elsinoe* infections. Plants were then placed in a glasshouse for eight weeks.

Results and discussion
Eleven of twelve nurseries surveyed stocked South African Proteaceae at the time of trial establishment, and in six of these nurseries plants were found with visible *Elsinoe* symptoms (54%). Some of the nurseries had a very high disease level on a number of plants.

Twenty-four symptom-free plants were bought from six of the nurseries surveyed and after eight weeks, four of these plants had developed Elsinoe scab. This showed that if symptom-free plants were exposed to increased humidity, temperature and moisture conditions such as in a polyhouse system, and then retained under normal glasshouse conditions, disease could develop if the plants had been
previously infected. The plants investigated in this trial may have become infected during propagation or later when placed in close proximity to other diseased plants in the retail nurseries.

The extent of latency is not clear, but some glasshouse trial plants have shown a delay in disease development of around eight weeks after inoculation. This delayed development of symptoms appeared to coincide with a drop in average glasshouse temperatures (ie. < 20°C) and a general reduction in humidity from 70-100% to 60-80% (although wide humidity fluctuations occurred in conjunction with mist watering).
6. Field Trial to Identify Environmental Conditions that Favour Spore Release from Elsinoe Affected Leucadendron Field Plants

Introduction
An important aspect of successful disease management is to identify the conditions that lead to spread of the disease (by spore dispersal and infection) and the progression of existing lesions. This will allow removal of diseased shoots prior to spore dispersal and application of fungicide sprays to prevent infection and further development of the disease.

Early field observations suggested that infection occurred only a few times during the year, presumably when temperature, humidity and rainfall levels were conducive to the formation and dispersal of spores and active plant shoot growth was occurring. To identify these critical periods, a field bait trial was conducted from February 1996 to February 1997 (detailed below) at a commercial property in Victoria.

Laboratory studies indicated that free water (such as rain or overhead irrigation) and high humidity were required for Elsinoe spore development, dispersal and infection of new plant material. Whiteside (1975) reported that E. fawcetti on Citrus sp. required water for production, dispersal and germination of conidia and minimum wetting periods of 2.5 to 3.5 hours for infection to occur. In studies of E. ampelina of grapevine in New Zealand, Brook (1992) commented that timing and quantity of rainfall may be critical for primary infection, but that rainfall in the order of 1-2 mm could promote further infection. Brook also suggested that earlier spring infections coinciding with warmer weather may be more a result of young shoot development and susceptibility, rather than a response of the fungus to increased temperature.

Objective
To identify environmental conditions that favour spore release from Elsinoe lesions of infested Leucadendron field plants.

Methodology

Trial design
Three Elsinoe-free varieties/selections were used as 'baits' to assess infection each fortnight. Comparisons were made on their susceptibility including at different positions within the host plant. The three varieties/selections of potted plants were placed in upper and lower positions of well-established and severely diseased host plants. Three replicate treatments were established in each of two rows. The rows were selected in separate areas of the same crop; one subject to the grower’s fungicide regime and the other in an area left unsprayed.

Plants and placement
Bait plant varieties/selections used in the trial were:

1. Leucadendron 'Silvan Red' and later 'Red Gem'
2. Leucospermum cordifolium
3. Serruria florida.

'Silvan Red' and 'Red Gem' were tested simultaneously for a number of weeks, to ensure that the change in the cultivar of the bait plants had no significant effect on the proportion of plants infected.
The host plants were *Leucadendron* sp. about 12 years old and heavily diseased with *Elsinoe*.

Bait plants in 14-cm pots were placed into metal holders on star pickets and set amidst foliage of diseased bushes at the trial site. This allowed spores from the host *Leucadendron* bushes to infect young bait plant shoots when weather conditions favoured spore development and dispersal.

**Assessment**

The 36 bait plants were collected fortnightly, exposed to high humidity in polyhouse systems to promote disease expression (Section 4.1) and assessed in subsequent weeks for presence or absence of lesions. The number of plants infected was recorded, but the degree of infection for each plant was not assessed. Fresh bait plants were placed in the field each fortnight and nine plants were retained as controls.

Preliminary assessment of plants from sprayed and unsprayed rows showed no difference in the number of bait plants diseased between these sites. It was subsequently found that fungicide spraying had ceased in the ‘sprayed’ area as the grower intended to replace these plants with other varieties. Moreover, following a number of deaths of host plants at one site, the trial was reduced to 18 bait plants per fortnight, under unsprayed conditions only.

**Results and Discussion**

Pooled data showed that peak infection periods occurred from February to mid-May 1996 and from mid-October 1996 through to early February 1997 (Figure 6.1). A relatively low number of bait plants became infected from late May to early August. Results indicated that on a property with high levels of disease, *Elsinoe* spores were present throughout most of the year and were able to infect young plant material.

![Figure 6.1: Bait plants infected with *Elsinoe* each fortnight at the Victorian trial site](image)

There was no significant correlation between the percentage of plants infected and temperature or rainfall values. Interaction of different environmental factors however, may produce combined conditions that favour spore dispersal and infection, for example, a minimum rainfall period coupled with a specific temperature range.

Data in figures 6.1 and 6.2 indicate that although there was no significant correlation between temperature and the number of plants infected, there is a general trend suggesting increased infection with increased temperature.
Infection rates were low or nil for temperatures below 20°C, suggesting that a threshold of 20°C may exist below which the potential for spore infection is greatly reduced. Where infection did occur at the lower temperatures (Figure 6.1 & 6.2), this appeared to coincide with the early plant growth in spring. It is speculated that the pressure of high host-plant spore levels on the very soft young growth of the bait plants at these times, caused infection to occur despite temperatures falling below 20°C.

**Figure 6.2** Fortnightly temperature data

*Maximum and, minimum temperatures for each two-week period (extracted from Bureau of Meteorology records)*

For each assessment fortnight at least some rainfall occurred; 6.0 mm was the lowest recorded (February 1997) and 131.6 mm was the highest recorded (August 1996) in any one fortnight. As discussed earlier, free water is required for spore dispersal and primary infection of new plant material. Bait plant infection and rainfall data (Figures 6.1 & 6.3) show that in many cases infection increased within three weeks of increased rainfall periods. Exceptions to this were where maximum temperatures fell below 20°C.

**Figure 6.3** Fortnightly rainfall data

*Rainfall data is displayed by maximum and total rainfall in each two-week period (extracted from Bureau of Meteorology records)*

**Figure 6.4** Percentage infected per variety

**Figure 6.5** Percentage infected (position in host bush)
Spore dispersal - upper and lower bush regions

In general, field observations have found heavier disease levels to occur in lower regions of well-established Proteaceous plants. Results from this experiment however, show that there was no difference between the percentage of bait plants infected, when placed in upper or lower regions of the host bush (Figure 6.5). This may suggest that:

1. some of the lesions lower in the bush are too old to produce viable spores, or
2. spore concentrations were similar throughout the plant due to splash dispersal.

7. Susceptibility of South African and Australian Native Proteaceae

Introduction
The survey conducted by IHD in 1994/5 found that many growers were still unaware of Elsinoe scab or unable to identify symptoms of the disease. This was perceived to be the primary cause for spread of the disease throughout the eastern coast of Australia. Understanding the existing and potential host range of the Elsinoe species affecting South African Proteaceae is important for on-property management and in preventing further spread of the disease. Initial field investigation indicated that species and varieties within the same genera varied in their susceptibility to Elsinoe.

Elsinoe scab has been confirmed on Protea species in South Africa, Zimbabwe, California and Australia (Swart et al. in press) and there is an indication that commercial losses of Protea are being experienced in Zimbabwe. There have been isolated reports of Elsinoe scab on Protea sp. and Mimites cucullatus in Australia and a native Elsinoe has been found on Banksias.

Isolates from Serruria, Leucadendron and Leucospermum were described by Swart et al. (in press) as representing Elsinoe leucoxpermi L. Swart and P. Crous. This species is the same Elsinoe as found on Leucospermum and Leucadendron hosts in South Africa (Swart et al. in press). Morphological examination of an Australian isolate from Protea obtusifolia in culture suggests that this strain may also belong to the same species. Some growth pattern differences (in culture) were observed for a Queensland isolate from Leucospermum, but this was insufficient evidence to suggest that it represented a different species. An isolate from Banksia prionotes was described as representative of Elsinoe banksiae I. Pascoe and P. Crous (Swart et al. in press).

The study discussed here showed that Elsinoe strains isolated from South African hosts in Australia can infect at least some Protea species, Mimites cucullatus, several native Banksia species and
**Objective**

To assess the susceptibility of 22 species and cultivars of Australian native and South African Proteaceae to infection by four strains of *Elsinoe* isolated in Victoria.

It was initially intended to rank major genera of Proteaceae, but in light of differences of susceptibility observed within genera and isolation of a number of *Elsinoe* strains, plants were selected to represent a broad range of commercially grown crops and for variation in susceptibility to fungal pathogens in general.

**Methodology**

**Trial design**

The trial was laid out on eight double-benches or "blocks", four on the west side of the glasshouse and four on the east. On the west side, each block comprised 96 plants set out in a 12 x 8 rectangle, the shorter side running parallel to the side wall of the glasshouse. The 88 plant variety by isolate combinations or "treatments" were randomised to positions, but structured so that all the treatments occurred once within each block (a complete "replicate"), and once within each of the four 32 x 3 rectangles of plants running parallel to the wall. The latter restriction was to ensure that any variation in conditions caused by the cooling units would affect treatments as equally as possible. Additional plants from the negative control “treatments” (ie. nil *Elsinoe* inoculum applied) were randomly allocated to the 8 remaining spaces in each block.

On the East side, each block comprised either a 15 by 8 rectangle of plants or a 14 by 8 rectangle. There were 110 treatments altogether, consisting of the negative controls for each of the 22 varieties, and the 88 variety by isolate combinations. The randomisation of treatments to positions had the same structure as the west side, except that each treatment occurred in four of the five 32 by 3 rectangles, again ensuring that the treatments were spread adequately in both directions. Remaining negative controls were randomly allocated to the 24 remaining spaces on the four benches.

In total there were eight replicates of each variety by isolate combination, and at least four replicates of the negative controls for each variety. The number of negative controls varied for the different plant varieties, and any in excess of four were randomly allocated to fill the spaces on the benches, but ensuring that the fifth from each variety (where available) was used before allocating the sixth from any variety, and so on.

**Plants**

Plants were obtained from four different suppliers in two and five centimetre tube pots. The former were re-potted into five centimetre tubes using "Debco" propagation mix. Suppliers had propagated the plants from seed or cuttings (dependent on plant varieties) and most plants were propagated between five and nine months prior to inoculation (nursery supplied information). *Grevillea australis* and *Hakea multilineata*, however, were propagated approximately 19 months earlier. The mentioned times include a 13-week 'rest' period in the research glasshouse for all varieties/cultivars, to ensure freedom from fungicide residues and disease prior to inoculation. Table 7.1 shows the 22 plant varieties and cultivars chosen.

Benches and watering systems were sterllised using a commercial preparation of 6.0% w/v hydrogen peroxide, 10.0% W/v ethanol and 0.25% w/v triclosa. This was sprayed directly onto benches and floors, left for five minutes and then removed using a high pressure hose system (Kärcher HD 850 WS). Bench sprinklers were placed approximately 60 cm apart and blocks were spaced 60-70 cm between benches with 70-100 cm between east and west sides. Plants were spaced sufficiently to ensure that no foliar contact occurred with adjacent plants, thus guarding against cross contamination.
Table 7.1 Proteaceae species and cultivars tested for susceptibility to infection with four *Elsinoe* isolates

<table>
<thead>
<tr>
<th>South African Proteaceae</th>
<th>Australian Native Proteaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Protea</em> ‘Pink Ice’</td>
<td><em>Telopea</em> ‘Red Shady Lady’</td>
</tr>
<tr>
<td><em>Protea cynaroides</em></td>
<td><em>Banksia prionotes</em></td>
</tr>
<tr>
<td><em>Protea obtusifolia</em></td>
<td><em>Banksia baxteri</em></td>
</tr>
<tr>
<td><em>Leucadendron salignum</em> ‘Red’</td>
<td><em>Banksia burdetti</em></td>
</tr>
<tr>
<td><em>Leucadendron</em> ‘Safari sunset’</td>
<td><em>Banksia coccinea</em></td>
</tr>
<tr>
<td><em>Leucadendron</em> ‘Highlights’</td>
<td><em>Dryandra formosa</em></td>
</tr>
<tr>
<td><em>Leucospermum pattersonii</em></td>
<td><em>Grevillea barklyana</em></td>
</tr>
<tr>
<td><em>Leucospermum cordifolium</em></td>
<td><em>Grevillea australis</em></td>
</tr>
<tr>
<td><em>Serruria florida</em></td>
<td><em>Grevillea pinaster</em></td>
</tr>
<tr>
<td><em>Mimites cucullatus</em></td>
<td><em>Hakea pincushion</em></td>
</tr>
<tr>
<td></td>
<td><em>Hakea salicifolia</em></td>
</tr>
<tr>
<td></td>
<td><em>Hakea multilineata</em></td>
</tr>
</tbody>
</table>
Isolates

_Elsinoe_ species isolates were cultured from Potato Dextrose Agar (PDA) slants covered with paraffin and isolate 1698 from a distilled water culture. All isolates were obtained from the National collection of fungi, Knoxfield Herbarium (VPRI) and grown on PDA plates. Isolate and host information is as follows:

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Host material</th>
<th>Date isolated</th>
<th>Herbarium number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1610</td>
<td><em>Leucospermum cordifolium</em> leaf and stem</td>
<td>8th August 1996</td>
<td>VPRI 21187 a</td>
</tr>
<tr>
<td>1612</td>
<td><em>Serruria florida</em> leaf and stem cankers</td>
<td>8th August 1996</td>
<td>VPRI 21188 a</td>
</tr>
<tr>
<td>1613</td>
<td><em>Banksia prionotes</em> leaf and stem lesions</td>
<td>5th August 1996</td>
<td>VPRI 21100 a</td>
</tr>
<tr>
<td>1698</td>
<td><em>Protea obtusifolia</em> leaf and stem material</td>
<td>13th November 1997</td>
<td>VPRI 21638 a</td>
</tr>
</tbody>
</table>

Inoculation

One or two colonies of each isolate were selected and grown on 9-cm PDA plates. When well established at around 12-14 days, one to two colonies from each culture were selected, crushed in two ml sterile distilled water and spread across two PDA plates. Colonies from these plates were further cultured until eight plates were covered with healthy _Elsinoe_ colonies for each isolate. At 10-16 days growth on the final plates, colonies were scraped from the PDA medium into 250 ml of sterile distilled water and blended at 4000 revs for 30 seconds using a Sorvall omni-mixer. One full plate of _Elsinoe_ colonies was used for each replicate of each isolate. The inoculum was transferred to 500-ml plastic household sprayers. Separate sprayers were used for each isolate and laboratory equipment was sterilised with 70% ethanol for each use. Approximately seven ml of inoculum was sprayed onto the soft new shoot material of each plant and the same quantity of distilled water was used for control plants. To ensure that any resultant infection was due to the pathogenic nature of the fungus, plants were not wounded or intentionally stressed.

Thirty minutes after application of the inoculum, plants were placed in a bench-top polyhouse (refer section 4.1) for 48 hours to induce infection. Light misting (20L per hour) was scheduled for two minutes three times a day within the polyhouses and the plastic was loosened after 24 hours, but only removed at 48 hours. Five polyhouses were used, allowing segregation of negative controls and plants inoculated with each individual isolate.

For each 48-hour period, one replicate block was inoculated, put through a polyhouse treatment and then allocated to positions on respective benches as per trial design. The inoculation of all plants therefore took sixteen days to complete. Average relative humidity values for the polyhouses ranged from 75% to 90%. Minimum and maximum temperatures recorded were 17.7°C and 41.09°C respectively, with an average of approximately 25°C for the five polyhouses. Tables 6.2 and 6.3 show that the widest range of values occurs within blocks (replicates of all treatments) and no significant differences were found between blocks. This showed that any variation in temperature and humidity values between replicates had little or no effect on disease incidence and severity.

**Table 7.2** Trial polyhouse humidity conditions

![](image)
Table 7.3 Trial polyhouse temperature conditions

<table>
<thead>
<tr>
<th>Treatment replicate</th>
<th>Av. Temp. (°C)</th>
<th>Min. Temp. (°C)</th>
<th>Max Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.23</td>
<td>18.43</td>
<td>39.89</td>
</tr>
<tr>
<td>2</td>
<td>22.74</td>
<td>17.77</td>
<td>33.46</td>
</tr>
<tr>
<td>3</td>
<td>23.62</td>
<td>18.09</td>
<td>41.09</td>
</tr>
<tr>
<td>4</td>
<td>24.79</td>
<td>17.77</td>
<td>37.06</td>
</tr>
<tr>
<td>5</td>
<td>25.80</td>
<td>17.77</td>
<td>38.16</td>
</tr>
<tr>
<td>6</td>
<td>26.27</td>
<td>18.76</td>
<td>37.61</td>
</tr>
<tr>
<td>7</td>
<td>24.23</td>
<td>19.09</td>
<td>35.47</td>
</tr>
<tr>
<td>8</td>
<td>25.66</td>
<td>18.09</td>
<td>36.52</td>
</tr>
</tbody>
</table>

Following the polyhouse treatment, plants were transferred to glasshouse benches and maintained under semi-controlled conditions. Recorded relative humidity values ranged from 59% to 98% with an average of 78% over the trial period. Minimum and maximum temperature values were 14°C and 23°C, with an overall average of 19.4°C. Watering was not commenced until approximately six hours after plants were transferred from each polyhouse, thus allowing any inoculum on the surface of shoot material to dry. This was aimed at restricting disease development to infection induced within the polyhouse system and reducing the likelihood of cross contamination. The second stage of possible contamination only arose when lesions developed on shoot material and released new spores. Expression of this type of secondary contamination would have been post-assessment and is therefore not relevant.

Assessment

Plants were assessed for disease incidence and severity from four to twelve weeks after inoculation. A total of 159 plants were visually determined as infected with *Elsinoe* and microscopically confirmed where necessary. Incidence was recorded as a proportion of the eight plants available for each plant/isolate combination. For two positive treatments, the death of one replica plant was considered in statistical analysis. Re-isolation from lesions and morphological examination of the fungus was successfully conducted on 66% of infected plants, with only one infection resulting from an isolate not consistent with the inoculum used for the treatment in question.

Severity of disease was rated as:

1 - low level of disease; negligible visual or physiological damage
2 - visual disfigurement of plant and/or physical damage which may effect further growth
3 - high number of lesions; visual disfigurement and/or effect on plant growth

Statistical analysis

Disease incidence was analysed using a statistical model, which took into account the binary nature of the data (presence or absence of disease). Because the interaction between plant and isolate was
significant at P < 0.05, comparisons were made in the analysis between each variety/isolate combination or “treatment”. This enabled treatments to be placed into groups of similar susceptibility. Disease severity was analysed using an analysis of variance, which tested the main effects of plant and isolate and their interaction. Although the data were discrete, this provided an approximate least significant difference for comparing mean severity levels of treatments.

Results and discussion

Disease incidence and severity

The proportion of diseased plants for each isolate/plant combination is shown in table 7.4, where 1.00 is 100% of eight (or in two instances seven) plants. For comparisons on disease incidence for plant/isolate treatments a difference in value of 0.50 or greater is significantly different (P-value of 0.05).

Table 7.4 Proportion of diseased plants following inoculation with four *Elsinoe* isolates

<table>
<thead>
<tr>
<th>Species/variety</th>
<th><em>Elsinoe</em> isolate</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>L. cordifolium</em></td>
<td><em>S. florida</em></td>
<td><em>P. obtusifolia</em></td>
<td><em>B. prionotes</em></td>
<td>control</td>
</tr>
<tr>
<td>Protea ‘Pink Ice’</td>
<td>0.00</td>
<td>0.25</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Protea cynaroides</td>
<td>0.25</td>
<td>0.13</td>
<td>0.38</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>Protea obtusifolia</td>
<td>0.00</td>
<td>0.00</td>
<td>0.13</td>
<td>0.14</td>
<td>0.00</td>
</tr>
<tr>
<td>Leucadendron salignum ‘Red’</td>
<td>0.13</td>
<td>0.13</td>
<td>0.50</td>
<td>0.38</td>
<td>0.00</td>
</tr>
<tr>
<td>Leucadendron ‘Safari sunset’</td>
<td>0.88</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Leucadendron ‘Highlights’</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Leucospermum pattersonii</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Leucospermum cordifolium</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Serruria florida</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.13</td>
</tr>
<tr>
<td>Minites cucullatus</td>
<td>0.88</td>
<td>0.88</td>
<td>0.50</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>Banksia prionotes</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.88</td>
<td>0.13</td>
</tr>
<tr>
<td>Banksia baxteri</td>
<td>0.38</td>
<td>0.38</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Banksia burdetti</td>
<td>0.00</td>
<td>0.13</td>
<td>0.00</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>Banksia coccinea</td>
<td>0.00</td>
<td>0.25</td>
<td>0.00</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>Telopea ‘Red Shady Lady’</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Dryandra formosa</td>
<td>0.63</td>
<td>0.38</td>
<td>0.25</td>
<td>0.71</td>
<td>0.00</td>
</tr>
<tr>
<td>Grevillea barkliana</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Grevillea australis</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Grevillea pinaster</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Hakea pincushion</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Hakea salicifolia</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Hakea multilineata</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Assessment results need to be interpreted on the basis of specific plant and isolate interactions only and the comparisons of these. Some generalisations can be made for example, that four *Banksia* varieties included in the study became infected with one or more of the *Elsinoe* isolates used. It cannot be said, however, that one variety was more susceptible than another variety or that one isolate was more pathogenic than another.

This experimental work makes use of a limited number of isolates and plant varieties under a relatively narrow range of conditions. Interpretation of data therefore has some limitations and the following should be noted:
“Not infected” is valid only under trial conditions and for the isolates used; it does not prove that the Elsinoe isolate could not infect this species, variety or cultivar under a different set of conditions.

“Infected” means under trial conditions, which may be uncommon or not occur in the field situation.

A low level of infection was recorded on two negative control plants early in the establishment of the trial. Successful re-isolation and identification of specific isolates from a large percentage of diseased trial plants however, eliminated any concern that further cross-contamination had occurred.

In this discussion, Elsinoe inoculum isolated from South African host plants (Leucospermum cordifolium, Serruria florida and Protea obtusifolia) in Australia, are referred to collectively as exotic isolates in comparison to inoculum isolated from Banksia prionotes.

Six of the ten varieties of South African Proteaceae investigated were susceptible to infection with all three exotic isolates under trial conditions and eight were susceptible to infection with at least one exotic isolate.

Disease incidence for L. ‘Safari Sunset’, L. cordifolium and S. florida with all three exotic isolates was high and not significantly different within these plant/isolate combinations (Figure 7.1). Disease severity was also similar, with the only significant difference in severity of infection being between the S. florida/S. florida and the L. ‘Safari Sunset/P. obtusifolia plant and isolate combinations (Figure 7.3).

Disease incidence for M. cucullatus, when inoculated with isolates from L. cordifolium and S. florida was statistically similar to the above. Incidence was somewhat lower for the isolate from P. obtusifolia, but not significantly different to inoculation of M. cucullatus with the other two exotic isolates. One plant was infected when inoculated with the isolate from Banksia (Figure 7.1). The 1994/5 survey reported only one commercial M. cucullatus crop affected by Elsinoe scab (Pascoe, Ziehrl and Porter 1995). The results of this trial however, demonstrate the potential for an increase in incidence of the disease on M. cucullatus, under favourable environmental conditions and the ability of this variety to harbour and spread the disease. Severity of disease on affected M. cucullatus plants is significantly less than for L. cordifolium and S. florida, but similar to L. ‘Safari Sunset’.

Disease incidence and severity on L. salignum ‘Red’ were significantly less for each exotic isolate than that caused by the same isolates on L. ‘Safari Sunset’. Unlike L. ‘Safari Sunset’ however, three plants also developed disease when inoculated with the isolate from Banksia.

Interestingly, L. ‘Highlights’ and L. pattersonii did not become diseased when inoculated with any isolate. The 1994/5 industry survey of Elsinoe affected crops (Pascoe, Ziehrl and Porter 1995) also cites no reported case of Elsinoe on either cultivar or species. Leucospermum crops on affected properties generally display severe disease symptoms, however a literature review did not show any confirmed cases of Elsinoe on L. pattersonii, but only on hybrids or on L. cordifolium. In one reported case of Elsinoe scab on L. pattersonii in Victoria, it was found that the plants were propagated from field stock and may have been hybrid.
Protea ‘Pink Ice’ developed disease on only two plants inoculated with the isolate from *S. florida* (notably not when inoculated with the isolate from *Protea*). *P. cynaroides* showed a low level of infection for inoculation with all exotic isolates, as well as the isolate from *Banksia*. A low level of disease was also determined for *P. obtusifolia* plants when inoculated with the isolates from *P. obtusifolia* and *Banksia*. Severity however, was low for all infected *Protea* plants.

Elsinoe scab has been confirmed on *Protea* species in South Africa, Zimbabwe, California and Australia (Swart *et al.* in press) and there is an indication that commercial losses of *Protea* crops are being experienced in Zimbabwe. Despite a general low severity of disease on *Proteas* in this trial, this raises concern about spread of the disease by these hosts and increased commercial loss to the Australian industry. It also highlights the need for strict hygiene and quarantine measures on commercial properties.

All *Banksia* species developed disease symptoms with at least one of the three exotic isolates (Figure 7.2). Incidence was significantly less than for inoculation with the same isolates on *L. ‘Safari Sunset’, L. *cordifolium* and *S. florida* (except for the *L. cordifolium* isolate/*L. ‘Safari Sunset’* treatment) (Figure 7.3). For inoculation with each of the three exotic isolates, fifty percent *B. prionotes* plants became diseased. Disease incidence for *B. prionotes* when inoculated with the *B. prionotes* isolate was comparable to incidence on *L. ‘Safari Sunset’, L. *cordifolium* and *S. florida* inoculated with exotic isolates.

*Dryandra formosa* became infected with all isolates and the incidence was recorded in two to five of eight plants for each treatment. No disease incidence was recorded for *Telopea* ‘Red Shady Lady’, *Grevillea* spp. or *Hakea* spp. (Table 7.5).
Figure 7.2 Proportion of diseased native Australian Proteaceae following inoculation with four *Elsinoe* isolates

Figure 7.3 Average severity rating of *Elsinoe* scab on affected trial plants

The least significant difference for comparing severity of disease means is 0.54 which is significant at $P = 0.05$.

Table 7.5 Diseased plant species and cultivars after inoculation with *Elsinoe* isolates
<table>
<thead>
<tr>
<th>Nil infection with any <em>Elsinoe</em> isolate</th>
<th>Infection with at least one <em>Elsinoe</em> isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leucadendron</em> ‘Highlights’</td>
<td><em>Protea</em> ‘Pink Ice’</td>
</tr>
<tr>
<td><em>Leucospermum pattersonii</em></td>
<td><em>Protea</em> cynaroides</td>
</tr>
<tr>
<td><em>Telopea</em> ‘Red Shady Lady’</td>
<td><em>Protea</em> obtusifolia</td>
</tr>
<tr>
<td><em>Grevillea barklyana</em></td>
<td><em>Leucadendron salignum</em> ‘Red’</td>
</tr>
<tr>
<td><em>Grevillea australis</em></td>
<td><em>Leucadendron</em> ‘Safari sunset’</td>
</tr>
<tr>
<td><em>Grevillea pinaster</em></td>
<td><em>Leucospermum cordifolium</em></td>
</tr>
<tr>
<td><em>Hakea pincushion</em></td>
<td><em>Serruria</em> florida</td>
</tr>
<tr>
<td><em>Hakea salicifolia</em></td>
<td><em>Mimites cucullatus</em></td>
</tr>
<tr>
<td><em>Hakea multilineata</em></td>
<td><em>Dryandra formosa</em></td>
</tr>
<tr>
<td></td>
<td><em>Banksia prionotes</em></td>
</tr>
<tr>
<td></td>
<td><em>Banksia baxteri</em></td>
</tr>
<tr>
<td></td>
<td><em>Banksia burdetti</em></td>
</tr>
<tr>
<td></td>
<td><em>Banksia coccinea</em></td>
</tr>
</tbody>
</table>

This trial, although conducted under semi-controlled conditions, demonstrates that the pathogenic potential of *Elsinoe* exists on these hosts. It is therefore important to consider:

- species and cultivars which may be involved in spread of the disease
- the potential risk of further commercial crop losses
- the need for rigorous hygiene measures and quarantine of new plant material
8. Fungicide Trials

8.1 Fungicide efficacy

Introduction
At February 2000 there were no fungicides registered specifically for use on Elsinoe scab of Proteaceae in Australia, but protea growers have been applying fungicides off-label to control the disease. The 1994/5 survey of the industry (Pascoe, Ziehrl and Porter 1995) showed that only 19% of growers believed chemicals were providing good control of the disease. Possible reasons for this include non-removal of diseased material, inadequate spray coverage, use of ineffective fungicides and inappropriate timing of spray applications (eg. only when disease symptoms have become severe). Fungicide application all-year-round by some growers achieved effective control, but was found to be uneconomical.

Elsinoe spore dispersal is associated with rainfall or overhead irrigation and appears to be most prevalent in the warmer months of the year (Section 6) when primary shoot development occurs and young soft plant material is more susceptible to infection. Removal of affected material, good hygiene practices and protection of new shoot material is critical before and during these periods.

In a seven-month field trial in Hawaii, researchers N. Nagata and S. Ferreira (Protea Management Group 1995 draft Newsletter) studied the efficacy of several fungicides for the control of *Elsinoe* on *Leucospermum* (hybrid). This research showed that all fungicides used gave good control, the three most effective being “Captan® (50WP)”, “Daconil® 2787 (40F)” (active chlorothalonil as in “Bravo® 500” used in Australian trials) and “Benlate® (50WP)” (active benomyl), in order of decreasing efficacy. Some phytotoxicity occurred with the use of Daconil® in an earlier greenhouse experiment (Protea Management Group 1993).

Objectives
The aims of the field trial in Australia were to:
1. determine the efficacy of a number of fungicides, including several currently being used by the Australian industry, as well as others tested in Hawaii
2. compare the efficacy of systemic and non-systemic fungicides, and
3. assess the appropriateness of each fungicide for disease control with regard to mode of action, persistence, phytotoxicity and cost.

Methodology

Trial design
The trial was established using five complete blocks within rows of a commercial *Leucadendron* plantation. Two blocks were assigned within each of two rows, and one block in a third row. Within each block, the treatments were randomised to plots of three adjacent plants. Nine fungicide treatments were compared with an untreated control, with 15 plants per treatment.

Plants
Plants used were *Leucadendron* “Silvan Red” and were approximately four years old at the start of the trial. Plants were irrigated as required, but some plants were lost during hot dry weather.

Pathogen
*Elsinoe* lesions were present on stems and leaves of all plants inspected prior to conducting the trial and although the disease was not severe, these lesions allowed for sufficient inoculum for on-going within-plant infection of actively growing shoots.
The ten treatments applied and their properties are presented in the following table.

### Table 8.1 Treatments used in trial work and fungicide properties

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Active ingredient</th>
<th>Mode of action</th>
<th>Trial application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>nil, water only</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>“Bravo® 500”</td>
<td>500g/L chlorothalonil</td>
<td>protective, non-systemic</td>
<td>150 ml per 100L water</td>
</tr>
<tr>
<td>“Dithane® M-45” fungicide</td>
<td>800 g/Kg mancozeb (alkylenebis dithiocarbamate)</td>
<td>protective, non-systemic</td>
<td>170 g per 100L water</td>
</tr>
<tr>
<td>“Bravo® 500” and “Dithane M-45” rotation</td>
<td>(as above)</td>
<td>(as above)</td>
<td>(as above)</td>
</tr>
<tr>
<td>“Shirlan®” fungicide - used on Brassica crops as a drench</td>
<td>500g/L fluazinam (2,6-dinitroaniline)</td>
<td>protective, slightly systemic and curative, good residual, good rainfast</td>
<td>100ml per 100L water</td>
</tr>
<tr>
<td>“Octave® WP” fungicide</td>
<td>462g/Kg prochloras</td>
<td>protective, eradicant</td>
<td>300g per 100L water</td>
</tr>
<tr>
<td>“Benlate® fungicide WP”</td>
<td>500g/kg benomyl</td>
<td>protective, curative, systemic</td>
<td>50g per 100L water</td>
</tr>
<tr>
<td>“ICI Captan® WDG” fungicide</td>
<td>800g/Kg captan</td>
<td>protective, curative</td>
<td>125g per 100L water</td>
</tr>
<tr>
<td>“Foli-R-Fos® 200” fungicide</td>
<td>200g/L phosphorous (phosphonic acid)</td>
<td>activates/enhances plant defenses</td>
<td>500ml per 100L water</td>
</tr>
<tr>
<td>“Sumisclex® 275 Flocol” liquid fungicide</td>
<td>275g/L procymidone</td>
<td>protective, curative, systemic</td>
<td>150ml per 100L water</td>
</tr>
</tbody>
</table>

The wetting/spreading agent “Agral® 600” was added to all treatments. This product was chosen because of specific recommendations for use with two of the fungicides and its compatibility with the other fungicides and a wide range of chemicals.

All chemicals were applied as foliar spray, including Shirlan®, which is specifically registered as a seedling drench for clubroot on Brassicas. Withholding periods were adhered to prior to any harvesting and where no recommendation was available for ornamentals, the period for the fruit or vegetable crop was used.

### Application

Foliar sprays were applied using a 15 L Birchmeier manual pump backpack sprayer. Approximately 500 ml of fungicide was sprayed on each plant to achieve full foliar coverage to pre-runoff. Rates were calculated from label recommendations (per litre or per hectare), to meet the coverage requirement per plant. Treatments were applied regularly every 14 to 28 days over two and a half years.

### Spray program

Fungicide product labels often recommend application every 14 to 21 days or as required, and the aim for this trial was to apply treatments every 14 days. In some instances, weather conditions and/or unforeseen circumstances resulted in application of fungicides at 21 to 28 days, which was not considered ideal for effective control.
Disease assessment

To obtain data for disease levels on commercial flower (marketable) shoots as well as on shoots that can harbour the disease from one season to the next, 15 flower shoots and 15 small vegetative shoots were assessed for each of the 150 trial plants. For each shoot, stems and leaves were separately rated for percentage of disease and disease distribution. In total, 18000 individual records were used in the data analysis. Ratings used to assess percentage area diseased and distribution of disease on Leucadendron plants are those described in section 4.4 and figure 4.1.

Briefly, the approximate mean percentage of diseased area on either the leaves or stems chosen for assessment, were rated as follows:

<table>
<thead>
<tr>
<th>Area diseased</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>0.5 % or less</td>
<td>1</td>
</tr>
<tr>
<td>1.0%</td>
<td>2</td>
</tr>
<tr>
<td>5.0%</td>
<td>3</td>
</tr>
<tr>
<td>10.0% or more</td>
<td>4</td>
</tr>
</tbody>
</table>

In addition, the disease pattern was also recorded at each assessment (refer to figure 4.1 for diagrammatic representation).

Shoots were selected at random from each plant and assessments were made on a 15-cm section of new growth, 5 cm below the base of the flower bud.

Data analysis

Data analysis was conducted on:
- percentage of surface area diseased, for both stems and leaves of each shoot assessed
- incidence of disease; ie. the number of plants where at least one Elsinoe lesion occurred on one or more of the 15 vegetative or flower shoot sections assessed
- number of shoots, where 5% or greater of the assessed surface area was diseased
- number of shoots where the pattern of disease represented only a few infection points (‘a’)

Each of these variables was subject to analysis of variance. Where necessary, an angular or logarithmic transformation was applied to stabilise the variance. Pairs of treatment means were compared using the least significant difference at P = 0.05.

Results and Discussion

Fungicide treatments for the control of Elsinoe scab on flower (marketable) shoots

Results showed that disease on marketable (flower) shoots of Leucadendron “Silvan Red” primarily occurred on the stem area. All chemical treatments applied achieved some level of control on flower shoots and efficacy comparisons for different aspects of the assessment are detailed below. Octave®, Captan® and Dithane® achieved a 75% reduction of disease on the surface area of Leucadendron flower stems and also significantly reduced the number of severely affected stems of flower (marketable). Diseased material was not removed prior to fungicide application and minimal attention was given to hygiene measures. It is therefore anticipated that much better disease control can still be achieved.

i) Effect of fungicide treatments on the disease level of flower shoots

Mean disease levels on the stems of flower shoots are given for each treatment in figure 8.1. The analysis showed that treatment effects were significant at P < 0.001. All treatments except Benlate®
demonstrated a significant (P < 0.05) reduction in disease levels. Benlate® also reduced disease, but less significantly (0.05 < P < 0.10).

Fungicide treatments appeared to fall into three main groups of efficacy:

<table>
<thead>
<tr>
<th>Group</th>
<th>Fungicides</th>
<th>Av. area diseased (approx.)</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Benlate®, Bravo®, Bravo®/Dithane®, Foli-R-Fos®</td>
<td>approx. 1-1.5 %</td>
<td>fair</td>
</tr>
<tr>
<td>B</td>
<td>Sumisclex®, Shirlan®</td>
<td>0.85 – 0.9%</td>
<td>good</td>
</tr>
<tr>
<td>C</td>
<td>Octave®, Captan®, Dithane®</td>
<td>less than 0.7%</td>
<td>very good</td>
</tr>
</tbody>
</table>

Mean disease levels for Sumisclex® and Shirlan® (group ‘B’) and Octave® (group ‘C’) were not significantly different to each other at P = 0.05, but with less than 0.7% disease on stem area, Octave® is more appropriately grouped with Captan® and Dithane®.

Treatment effects discussed here relate to the diseased stem area of marketable (flower) shoots. A reduction of diseased area from 3% to less than 0.7% (group ‘C’ fungicides) translates into a 75% reduction of visual disease symptoms which directly enhanced the marketability of these stems.
Disease levels on the surface area of the leaves of flower shoots (as opposed to stem regions), were low for all treatments (< 1.5%) including the control, and there was little difference between treatments (Figure 8.2). These results indicate that the leaves of *Leucadendron* “Silvan Red” were not as susceptible to infection by *Elsinoe* as the stems.

**Figure 8.1** Elsinoe scab disease levels on stems of flower shoots
LSD (P = 0.05) 0.44

**Figure 8.2** Elsinoe scab disease levels on leaves of flower shoots
LSD (P = 0.05) 0.14

**ii) Effect of fungicide treatments on the number of severely diseased stems and leaves of flower shoots**

The number of severely diseased stems for each treatment was also compared. This was arbitrarily selected as being stems with lesions on 5% or more of the surface area assessed (rating 3 or greater). The data required angular transformation, as percentages fell close to zero and did not conform to a normal distribution. The LSD value from this transformation (8.92 at P = 0.05) was used for comparison of transformed means.

All treatments except Bravo® significantly (P < 0.05) reduced the number of severely diseased stems (figure 8.3). Octave® and Dithane® reduced the mean percentage of severely diseased stems from 15% to 0% and Captan® from 15% to less than 0.5%.
Figure 8.3 Percentage of flower shoots with at least 5% of area on stems affected by *Elsinoe* lesions (transformed data)

Severely diseased leaves were not recorded on flower shoots in the control treatment or in any of the fungicide treatments except for Bravo® (0.44% of shoots).

*iii) Incidence of Elsinoe scab on plants (assessed by flower shoots)*

The incidence of disease was also assessed, ie. the number of plants where at least one *Elsinoe* lesion occurred on one or more of the 15 flower shoot sections assessed per plant. It was found that greater than 90% of plants, for all treatments, possessed at least some evidence of the disease on new growth. This demonstrated the effective perpetuation of the disease once established within a crop and reiterates the importance of removal of diseased material.

*iv) Mode of action of fungicides in relation to infection by Elsinoe spores*

Data were also recorded on the disease pattern on flower shoots (refer figure 4.1 for details). A few large lesions were generally regarded as successful infection with a few spores, followed by considerable lesion development (rating ‘a’), whereas numerous small lesions signified infection by many spores with little further disease progression (rating ‘b’).

Figures 8.4 and 8.5 illustrate that for Octave®, Captan® and Dithane®, the percentage of stems and leaves with relatively few lesions in relation to surface area affected, was slightly higher overall than for most other treatments. This suggested that part of the efficacy of these fungicides, was due to their ability to protect against new infection. These non-systemic fungicides should therefore be applied prior to conditions conducive to spore release and infection of plants.
Outcomes for growers using any of these products

- be aware of label recommendations/limitations and State specific registration
- use rotation of fungicides to avoid pesticide resistance
- for all varieties and cultivars, test a small number of plants for phytotoxicity to fungicides
**Fungicide treatments for the control of Elsinoe scab on vegetative (non-productive) shoots**

Vegetative shoots on Proteaceae are small, soft and numerous and are of little importance to the grower in terms of marketability. They generally remain untouched on commercial plants from season to season and often carry well-advanced *Elsinoe* lesions, which can provide a large reservoir of inoculum for new infections. It is this ability of vegetative shoots that poses a direct threat to plant health and production of marketable stems.

**i) Effect of fungicide treatments on the disease level of vegetative shoots**

Results showed that disease on vegetative shoots, as for flower shoots, occurred primarily on the stem regions of the *Leucadendron* plants. The level of disease for both stem and leaf areas, however, was considerably higher than for flower shoots. All fungicide treatments provided a significant reduction of Elsinoe scab disease on stems and leaves of vegetative shoots except Sumislex®, which failed to reduce levels of the disease on leaves (Figure 8.6 & 8.7). Octave®, Captan® and Dithane® showed a similar trend of efficacy as on flower shoots, but the differences between fungicide treatments were generally not significant.

**Figure 8.6** Elsinoe scab disease levels on stems of vegetative shoots

LSD (P = 0.05) 0.47
ii) Effect of fungicides on the number of severely diseased stems and leaves of vegetative shoots

The percentage of vegetative shoots with severely diseased stem areas (5% or greater), was also greater than for flower shoots. All fungicide treatments except for Sumislex® provided a significant reduction of severely affected stems (Figure 8.8). Efficacy of these treatments was difficult to separate, although the lowest percentages were recorded for Octave®, Captan® and Dithane®.

iii) Incidence of Elsinoe scab on plants (assessed by vegetative shoots)

The incidence of disease per plant, as for flower shoots, was in excess of 90%. This reiterates the effective perpetuation of disease and the need to remove diseased material.

iv) Mode of action of fungicides in relation to infection by Elsinoe spores

Analysis of the disease pattern on both stems and leaves of vegetative shoots provided little incisive information. In general, mean disease levels and the number of severely affected shoots were much
higher than on flower shoots. Large lesions may therefore have formed from i) single spore infection sites or ii) the amalgamation of several small lesions.

**Outcome**

Elsinoe scab lesions remaining on plants provide an on-going source of inoculum for new infections. The high level of disease on vegetative shoots suggests that it may be more difficult to protect this soft material from infection and also highlights the need for growers to invest resources on removal of these shoots. This may also assist in spray penetration and fungicide efficacy.

### 8.2 Spray application

**Introduction**

Efficacy of spray programs relies not only on use of effective chemicals and correct timing of application, but also on spray distribution and droplet size. Chemical control of pathogenic fungi requires that good spray distribution be achieved throughout the crop, within individual plants and across leaf and stem areas.

The minimum recommended droplet distribution for insecticide spraying is much less than for fungicides (Ciba-Geigy Ltd 4th ed.). However, it was found that at least some Victorian growers used the same spray settings for insecticide and fungicide application and many did not adjust nozzle height or orientation, or tractor speed, when spraying crops with differing height or foliar characteristics. Uneven spray distribution may cause waste of product and/or ineffective control even within the same plant.

**Objectives**

To test the fungicide distribution of several different spray application procedures used by growers

1. within rows
2. within plants

**Methodology**

Water sensitive paper strips and accompanying instructions (Ciba-Geigy Ltd 4th ed.) were supplied to six Victorian growers. It was known that the type of spray equipment differed between growers, but in each case the equipment was considered by growers to be appropriate and effective for fungicide application. Each grower was asked to place these strips into Proteaceae plants just prior to the next fungicide spray application and that spraying be conducted in the usual manner. It was requested that these be dried and forwarded to IHD.

Two growers returned the test strips; two growers provided verbal feedback about the spray distribution and two growers did not complete the test procedure. Efficacy of spray application was determined for the four cases where feedback was supplied. Assessment of effective coverage was evaluated using the instructions accompanying the water sensitive paper (Ciba-Geigy Ltd 4th ed.).

**Results and Discussion**

Assessment of water-sensitive paper tests found uneven fungicide spray penetration and distribution for each of the four tests conducted on Victorian properties. One grower reported fair spray distribution and coverage, although some strips showed below recommended droplet density and run-off occurred on others. For the remaining three tests, distribution was uneven and droplet density ranged from less than 10 droplets per cm², to 100 percent coverage, ie. run-off.

Test strips showed that for critical sites, such as in lower thicker regions where disease can proliferate or higher regions where marketable stems are at risk, coverage was not complete and droplet density was inadequate for approximately 50% of the strips in these regions.
Working with one Victorian grower, it was found that coverage could be improved with adjustment of nozzle orientation, air volume output and tractor speed.

Growers need to be aware that spray application procedures are critical in ensuring the efficacy of fungicides and that droplet size and distribution recommendations differ for insecticides, herbicides and fungicides. On-farm testing of spray methods should be conducted together with equipment suppliers and/or agricultural service advisers.
9. Industry Implications

Research results discussed in this report present a range of implications for the Protea and Wildflower industries in relation to severity and spread of Elsinoe scab. Important aspects of the disease in Australian include: biology of the causative fungus, epidemiology of the disease, cultural practices, trade practices and fungicide efficacy and application techniques and plant growth flush times. An integrated approach to management of Elsinoe scab is required to minimise the severity and impact of the disease.

In this study, it was shown that on a property with high disease levels, *Elsinoe* spores may be present throughout most of the year. These spores can infect plants whenever susceptible material is present, such as in spring and autumn and when free water is available for spore germination. Data also indicated that a threshold of 20°C exists below which infection is greatly reduced. Thus, spore levels, availability of free water and moisture, temperature and growth flushes are important factors influencing infection and disease development. The interaction of these factors and possible disease outcomes are diagrammatically represented in figure 9.1.

![Diagram showing the interaction of temperature, moisture, plant growth stage, spore levels, and disease levels.](image)

**Figure 9.1** Potential infection levels in relation to temperature, moisture, plant growth stage and spore pressure
Spraying plants with Octave®, Captan® and Dithane® significantly reduced Elsinoe scab on flower shoots and reduced the number of severely diseased stems, thereby increasing the marketability of flower shoots and plant productivity.

A survey of retail nurseries trading in South African Proteaceae, indicated that more than 50% stocked diseased plants. This suggests that Elsinoe scab is currently being spread to new sites and plantations through the sale of diseased plants.

The variable susceptibility of South African Proteaceae, to infection by four different isolates of *Elsinoe*, may be a consequence of varied resistance by the varieties/cultivars or of the pathogenecity of the isolates under trial conditions, or a combination of these factors. It is interesting to note that no disease was observed on *L. ‘Highlights’* and *L. pattersonii* plants in the glasshouse trials. It is necessary to establish if these cultivars have field resistance.

Infection of *Banksia* spp. and *Dryandra formosa* by *Elsinoe leucospermi* isolates in our trials, should be of concern to both the Protea and Wildflower industries. If Elsinoe scab appeared on these commercial varieties, there is a possibility that the disease may become well established on native Australian flora and adversely affect production and sales.
## 10. Recommendations

### An integrated approach to the control of Elsinoe scab

A number of key issues were identified in this study as contributing to the economic loss attributed to Elsinoe scab and the spread of the disease throughout the Australian industry. These issues and some basic approaches and/or remedies are listed below.

<table>
<thead>
<tr>
<th>Issue</th>
<th>Management approach</th>
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<tbody>
<tr>
<td>1. Difficulty in disease identification</td>
<td>Contact a local agricultural advisory service</td>
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<tr>
<td>2. Trade of plants prior to symptom development after initial infection</td>
<td>Use only reputable suppliers</td>
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<td></td>
<td>Quarantine new stock for up to six months prior to planting, preferably with sprinkler irrigation to encourage symptoms of any existing disease</td>
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<tr>
<td>3. Splash dispersal of spores</td>
<td>Avoid overhead irrigation</td>
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<tr>
<td>4. Physical disease transfer on-property</td>
<td>Sterilise harvesting and pruning tools</td>
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<tr>
<td>5. Disease transfer through cuttings</td>
<td>Do not use diseased plants for cuttings</td>
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<tr>
<td>6. Perpetuation of existing disease</td>
<td>Prune out and destroy all infected material, paying particular attention to small non-productive shoots which have been shown to carry high levels of disease</td>
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<tr>
<td>7. Inadequate spray penetration and/or leaf stem coverage and droplet distribution</td>
<td>On-farm consultation and testing of spray methods (eg. with water sensitive strips), should be conducted together with equipment suppliers or other agricultural advisers</td>
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<tr>
<td></td>
<td>Pruning to allow greater spray penetration</td>
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<td>8. Close proximity of plants</td>
<td>Plan planting distances to allow maximum air circulation between mature plants</td>
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<td>Increased spacing between pots will reduce the risk of disease transfer</td>
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<td>9. Susceptible varieties</td>
<td>Use less susceptible cultivars and selections and/or conduct on-property selection trials for greater resistance to Elsinoe</td>
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<tr>
<td>10. Timing of spray application</td>
<td>Do not wait for symptoms to appear on new shoot material, apply fungicides when conditions and plant growth are likely to result in new infections (figure 10.1)</td>
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<td>Primary infection may allow secondary infection to occur throughout the season</td>
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<td>11. No fungicides registered for Elsinoe</td>
<td>Seek all available information on control</td>
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<td></td>
<td>Use fungicides within State registration and Label guidelines</td>
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<tr>
<td>12. High cost of fungicides</td>
<td>Time sprays carefully to obtain maximum control and minimise the number applied</td>
</tr>
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<td></td>
<td>Use cost effective fungicides</td>
</tr>
<tr>
<td>13. Plant losses due to fungicide toxicity</td>
<td>Test a few plants first for phytotoxicity</td>
</tr>
</tbody>
</table>
To effectively manage Elsinoe scab, the factors that influence primary infection of new plant material need to be considered. These may be very specific to the property or climatic region in question and relate to:

- the level of potential spore release from existing diseased plants on the property,
- available free water and moisture, and conditions of temperature and humidity in relation to the formation and release of spores, subsequent primary and secondary infections and the development of existing lesions
- the age and growth stage of plants when spores are released and infection potential is high

Figure 9.1 diagrammatically represents potential infection levels under different conditions and provides a guide to plant protection requirements. It is important to protect the very first growth of the season before any visible disease symptoms can be seen on the new shoots. Once primary infection has occurred, available moisture on these shoots may cause secondary infections and increase the size of existing lesions. Plant protection by fungicides may be required as early as mid-winter, dependent on seasonal temperatures and growth flushes.

The fungicides that provided the best control of disease on flower shoots viz. Octave®, Captan® and Dithane®, are non-systemic protectants and would require application prior to periods of rainfall and increased humidity and temperature, particularly at the onset of, and during, plant growth flush periods. Good spray application techniques are essential for the fungicides to effectively protect plant material. Initial removal of diseased material is strongly advised, particularly the small non-productive shoots that remain on the plant from season to season.

As plants may carry Elsinoe scab with or without visible symptoms, there is a need for the formulation and introduction of a comprehensive nursery hygiene program. This would help reduce spread of the disease and should include; education on identification of the disease, removal of diseased plants, improved airflow, wider pot spacing and reduced humidity. It is recommended that industry members and research staff work together to develop and implement a nursery hygiene and education program.

The finding that Australian native Proteaceae can become infected with Elsinoe isolates from South African varieties, raises concern about the potential of new varieties to incur economic losses, or at the least harbour the disease and contribute to further spread. This may warrant a broad review of current hygiene and plant distribution practices within the Protea and Wildflower industries. It is also recommended that further research be conducted on the in-field susceptibility of different varieties.

**Further actions and dissemination of outcomes**

Provisions should be made for results, industry implications and recommendations detailed in this report, to be presented at forum for key stakeholders of the Protea and Wildflower industry, and include representatives such as growers, nursery people and exporters. This is seen as a means to foster discussion on the control of this disease and/or further research to be conducted by i) growers (eg. for resistant varieties) or ii) research institutes (eg. on the susceptibility of Australian native varieties and further development of disease prediction models).

This study has also indicated that growers may benefit greatly from industry field days, aimed at improving spray application techniques and with consideration of crop characteristics and the type of pesticides being applied.

It is important that the implications and recommendations of this report are disseminated widely within the Protea and Wildflower industries.
11. References


Whiteside, J.O. (1975). *Biological characteristics of Elsinoe fawcetti pertaining to the epidemiology of Sour Orange Scab*. Journal Series paper No. 5790, Florida Agricultural Experiment Station.

