Know-how for Horticulture™

Managing diseases of leeks

Catherine Hitch
SA Research &
Development Institute

Project Number: VG00013

VG00013

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry.

ISBN 0 7341 1147 9

Published and distributed by:
Horticultural Australia Ltd
Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

Know-how for Horticulture™

Managing Diseases of Leeks

FINAL REPORT

HORTICULTURE AUSTRALIA LIMITED

VG00013

By C.J. Hitch, E.A. Oxspring, T.J. Wicks, and B.H. Hall
South Australian Research and Development Institute

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Australian Government
Department of Agriculture, Fisheries and Forestry

Government of South Australia
Primary Industries and Resources SA

HAL
Know-how for Horticulture™

HORTICULTURE AUSTRALIA LIMITED

Final Report March 2005

Project title: Managing diseases of leeks

HAL Project no.: VG00013

Research Organisation: South Australia Research and Development Institute
GPO Box 397, Adelaide SA 5001
Contact: Trevor Wicks
Phone (08) 8303 9563 Fax (08) 8303 9393
Email: wicks.trevor@saugov.sa.gov.au

Principal Investigators: Catherine Hitch
Phone: (08) 8303 9587 Fax: (08) 8303 9393
Email: hitch.catherine@saugov.sa.gov.au
South Australian Research and Development Institute
GPO Box 397, Adelaide SA 5001

Liz Oxspring

Little is known about diseases of leeks in Australia. Although leeks belong to the Allium family and are likely to be prone to similar diseases that attack onions, data is scarce on the main problems affecting leeks in Australia. The aims of this project were to determine the main disease problems of leeks in Australia, develop management strategies to control these diseases and to ensure that this new information and technology is adopted by the industry.

We acknowledge the Vegetable industry and the Commonwealth Government for funding this project through Horticulture Australia.

The fungicides evaluated in trials during this project are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only and further trials are required to confirm best management practices.

CONTENTS

MEDIA SUMMARY

Leeks have become an important component of vegetable production in Australia with the area of production increasing in most states. Little is known about diseases of leeks in Australia. Although leeks belong to the Allium family and are likely to be prone to similar diseases that attack onions, data is scarce on the main disease problems in Australia. Diseases have been reported on commercial crops in Australia and the spread of these problems could jeopardise further development of the industry and affect the maintenance of existing domestic and export markets. The main objectives of this project were to identify the disease problems on leeks in the main production areas in Australia and to develop management strategies to control these problems.

Extensive surveys of leek plantings in Australia were undertaken in SA, Vic, WA and Qld. Two main diseases, Fusarium foot rot (*Fusarium avenaceum* and *F. oxysporum*) and Bacterial blight (*Pseudomonas syringae* pv. *porri*) were found to cause significant economic losses in plantings in most states. Leaf blight (*Stemphylium botryosum*), while widespread in all states, only caused cosmetic damage. Purple blotch (*Alternaria porri*) was found in Victoria and Queensland, and while severe infection can reduce marketability most damage was also cosmetic.

Other diseases found on leeks in Australia were Smudge (*Colletotrichum circinans*), Botrytis leaf spot (*Botrytis cinerea*), Pink root (*Pyrenochaeta terrestris*) and Oedema (caused by environmental conditions). Viruses including Leek Yellow Stripe, Shallot Latent and Onion Yellow Dwarf were found on leeks in Australia however they were not widespread. Other organisms found associated with infection from *Fusarium* and *Pseudomonas* included onion maggot and 3 species of parasitic nematodes.

Surveys also showed that seedlings can often be infected with *Fusarium* without showing symptoms. All commonly planted varieties including Nova, Admiral, Missile, Tokyo and Harpoon were susceptible and trials showed that planting infected seedlings increased seedling mortality and *Fusarium* infection at harvest. Laboratory and greenhouse trials were undertaken to evaluate the efficacy of fungicides on pathogenic *Fusarium* and those showing promise were further evaluated in field trials. Treating seedlings with fungicide drenches prior to planting, or fungigation treatments (irrigating with a fungicide solution after planting) controlled *Fusarium.* The unregistered fungicides Octave (prochloraz) applied as a drench to speedling trays prior to planting or Bavistin (carbendazim) applied as a fungigation treatment reduced *Fusarium* infection at harvest.

Bacterial blight caused by *Pseudomonas syringae* pv. *porri* was shown to be seed borne in leek seeds planted in Australia. Preliminary studies showed that soaking seed in hot water eliminated the bacteria from the seed, but the treatment severely reduced germination. Further investigations and strategies need to be developed to provide effective management strategies to reduce bacterial blight infection in leek crops. Research needs to be undertaken to find effective treatments to eliminate bacteria in seed while maintaining acceptable levels of germination. Investigations also need to be carried out to determine how the bacteria spreads in plantings and if copper sprays control the disease.

TECHNICAL SUMMARY

Leek plantings throughout Australia were surveyed for diseases from 2000-2003 to determine the pathogens effecting yield and marketability of leeks. The results of these investigations including pathogens, symptoms, variety susceptibility and control strategies are mentioned below in order of disease significance at the time of investigation.

1) Fusarium foot rot

- Caused by *Fusarium avenaceum* and *F. oxysporum.*
- Plants mainly develop pale yellow/brown or pink crown or basal rot.
- *Fusarium avenaceum* is the most common species pathogenic to leek seedlings and mature plants.
- Major disease problem in seedlings and mature plants in Australia.
- Optimum temperature for growth is 20-25^{o}C.
- Leek varieties common to all growing areas (including Harpoon, Missile, Admiral, Tokyo, Amundo and Nova) are susceptible to *Fusarium avenaceum.*
- Low infection levels on seedlings resulted in reduced infection at harvest.
- *In vitro* screening indicated that *Fusarium avenaceum* is inhibited by carbendazim and prochloraz.
- Field trials in South Australia and Victoria showed pre plant fungicide drenches and post planting fungigation treatments controlled *Fusarium.*
- *Fusarium* was controlled by applying either an prochloraz drench to seedlings or fungigating with carbendazim immediately after planting.

2) **Bacterial blight**

- Caused by *Pseudomonas syringae* pv. *porri*.
- Infection develops as brown leaf lesions surrounded by a yellow halo and as longitudinal water soaked tissue extending as a narrow strip from the leaf tip to the crown.
- Leaves are often curled, water soaked and light green in colour.
- Affects seedlings and mature plants in Australia.
- *Pseudomonas* is seed borne and infected plants will produce infected seed.
- Hot water treatments eliminate bacteria from seed but severely inhibit germination.

3) Leaf blight

- Caused by *Stemphylium botryosum.*
- Symptoms appear as pale oval lesions which turn brown after spore production.
- Damage is cosmetic in all growing areas of Australia causing both primary and secondary infection.
- Leek varieties common to all growing areas (including Harpoon, Missile, Admiral, Tokyo, Amundo and Nova) are susceptible to infection.
- Optimum temperature for growth is 25^{o}C.
- *In vitro* screening showed *Stemphylium* was inhibited by difenconazole and iprodione.

4) Purple blotch

- Caused by *Alternaria porri.*
- Symptoms appear as purple oval lesions which darken with spore production.
- In severe infections blotching extends down the stem of the leek causing serious cosmetic damage and plant death.
- Found in Victoria and Queensland.
- Optimum temperature for growth is 25^{o}C.
- *In vitro* screening showed *Alternaria* was inhibited by difenconazole and iprodione.

Various other diseases, disorders, viruses and organisms were found during the survey including:

- Smudge (*Colletotrichum circinans*)
- Botrytis leaf spot (*Botrytis cinerea*)
- Pink root (*Pyrenochaeta terrestris*)
- Oedema
- Leek yellow stripe, Shallot latent and Onion yellow dwarf virus
- Onion maggot
- Parasitic nematodes

LITERATURE REVIEW

An extensive literature review confirmed that little work has been done on leek diseases in Australia with most of the studies being done in America, Europe and the United Kingdom.

Two important leek diseases present overseas and not reported in Australia are rust caused by *Puccinia allii* (4, 10) and white tip caused by *Phytophthora porri* (10).

In America bacterial blight of leek caused by *Pseudomonas syringae* has been identified as a significant disease of leeks. The disease appears as water soaked, longitudinal lesions which start at the leaf tip and extend down the leaf (7). Symptoms similar to this have been observed on leek plants in South Australia and *Pseudomonas syringae* pv. *porri* has been identified from these plants.

A number of other bacteria have been associated with Alliums in Australia including *Erwinia carotovora* pv. *carotovora* associated with shallots and onions, *Pseudomonas aeruginosa*, *P. cepacia*, *P. gladioli* pv. *alliicola* and *P. marginalis* pv. *marginalis* in onions (3).

Leaf blight caused by *Stemphylium vesicarium* has been identified in garlic in Australia (12). This fungus causes white flecks which enlarge to produce sunken purple lesions occasionally surrounded by a yellow to pale brown border on garlic. Similar symptoms are found in leeks in South Australia.

In California purple blotch, caused by *Alternaria porri,* has been identified as a major pathogen in leeks (6). Another major pathogen in England and Ireland is *Cladosporium allii*, which causes leaf blotch diseases (5).

Studies on the effects and characterisation of viruses in Allium species including Onion Yellow Dwarf and Leek Yellow Stripe have been carried out in France (9) and the Netherlands (13).

Searches on the internet and data from diagnostic samples collected throughout Australia have found white rot (*Sclerotium cepivorum*), pink root (*Pyrenochaeta terrestris*), downy mildew (*Peronospora destructor*), botrytis leaf spot and neck rot (*Botrytis cinerea* and *B. allii*), Smudge (*Colletotrichum circinans*), and Fusarium foot rot (*Fusarium*) on leeks.

IDENTIFICATION OF DISEASES IN LEEKS

Introduction

The first objective of this project was to determine the main diseases of leeks in Australia. Leeks are an important component of vegetable production in Australia with areas of production increasing in most states. They have become more common in the domestic diet and export markets for the product have been established. However, little is known about the diseases affecting this crop in Australia, although the plant is related to onions. Diseases have been reported on commercial crops in Australia and spread of these problems could jeopardise further development of the industry and affect the maintenance of existing domestic and export markets.

Survey methods

Extensive surveys of leek plantings in Australia were undertaken throughout the first 2-3 years of the project. Vegetable Industry Development Officers were contacted in each state at the beginning of the project and growers informed of the project and properties visited. Three commercial plantings of leeks in South Australia located in the Adelaide Hills, Langhorne Creek and Murray Bridge were visited regularly from 2000 to 2003. Plantings in Cranbourne and Clyde in Victoria were visited on 2 occasions in 2001 and 2003, and properties at Wanaroo and Wattleup in Western Australia were visited in 2002. Samples were also received from one grower in Queensland and this property was visited in 2002. Nurseries were visited and sampled in Victoria. During the survey growers were encouraged to send in diseased plant material for identification.

The survey involved collecting diseased plant material from leek plantings at various growth stages, from different varieties and locations. Leeks showing disease symptoms were collected, forwarded to the laboratory and examined microscopically for evidence of fungi and bacteria before isolations were made onto various media. Over 600 fungi and bacteria were isolated from leek plantings throughout Australia. Isolates were tested for pathogenicity as many organisms are known to cause secondary infections. Pathogenic isolates were sent to mycologists and bacteriologists for further identification. Leaves with streaking or other virus like symptoms were frozen and tested for poty and carlavirus. Soil and potting mix was also collected and tested for presence of pathogens.

Isolation of organisms from diseased material

Fungi

Diseased tissue from the roots and crowns of leek seedlings and mature plants were surface sterilised using 4% sodium hypochlorite solution, rinsed thoroughly, dried in a laminar flow and plated onto Potato Dextrose Agar (PDA), Tap Water Agar (TWA) and Spezieller Nährstoffarmer Agar (SNA) (15) and incubated at 25^{o}C for 10-14 days with a 12 hour photo period.

Fungi consistently recovered from diseased crown and root sections of seedlings and mature plants developed *Fusarium* macro and micro conidia on TWA and SNA. Mycelium was generally white on TWA or tinged coral/peach on SNA. Conidia were more abundant on SNA. Pigmentation of the agar was pronounced on PDA with colours ranging from yellow to brown and pink to deep burgundy. Colony morphology, pigmentation and the presence of conidia were recorded.

Conidia from sporulating leaf lesions were removed using sticky tape and examined microscopically. Diseased leaf tissue from mature plants was also surface sterilised using 4% sodium hypochlorite solution, rinsed thoroughly, dried in a laminar flow and plated onto PDA and TWA and incubated at 25^{o}C for 2-3 weeks. Isolates of each fungi were sent to M. Priest, mycologist with New South Wales DPI at Orange for identification.

Bacteria

Diseased leaf and crown tissue suspected to be infected with bacteria was tested for bacterial streaming. A 5mm section of plant material was taken from the margin of infected tissue, placed in a drop of water on a microscope slide and examined microscopically for the presence of bacterial streaming. When present, the bacteria suspension was streaked onto Nutrient Agar (NA) and the selective media King's B (KB) and incubated at 28^{o}C for 48 hours. Plates were then examined under a UV (ultra violet) light to determine the presence of fluorescent *Pseudomonas*.

Bacterial streaming was consistently observed from brown leaf lesions, yellow streaking and crown tissue. Bacterial colonies developed when streaked onto NA and KB plates and isolates on KB plates fluoresced under UV light indicating the presence of the bacteria *Pseudomonas*.

Isolates were maintained on NA and sent to bacteriologists (Dr E Cother and Ms D Noble) with the New South Wales DPI at Orange for identification.

Pathogenicity

Fungi

Pathogenicity tests were carried out on over 355 isolates of *Fusarium* recovered from the crowns and roots of leek seedlings and mature plants by inoculating either leek seedlings or discs of leek leaf tissue. Pathogenicity of 5 *Stemphylium* and 5 *Alternaria* isolates recovered from leaves of mature plants were tested by inoculating mature leek leaves.

Materials and methods

Leek seedling assay

Leek seed cv. Amundo were sterilised by immersing for 3 minutes in 70% ethanol and drying in a laminar flow cabinet. Seeds were then sown in tubs containing a sterilised 50:50 perlite: vermiculite mix, watered and maintained in a growth room at 25^{o}C. *Fusarium* isolates were grown on SNA for 7-10 days and each plate macerated to inoculate five seedlings. In the initial tests, each 2-4 week old seedling was planted into a 5cm pot half filled with UC soil mix, covered with a 1cm layer of macerated *Fusarium* inoculum and filled with UC soil.

In later tests, 2-4 week old seedlings were planted 6 per pot and the soil inoculated by applying 1ml of a conidia suspension containing 10^{6} spores/ml to the base of each seedling. Pots in both methods were watered regularly and maintained in a greenhouse at 25^{o}C and assessed weekly for 3 months for dead and missing plants.

Leek disc assay

A quicker method to determine pathogenicity was developed using mature leeks of unknown cultivar purchased at the supermarket. Cut into 1cm thick discs and surface sterilised in a 4% hypochlorite solution for 3 minutes the leek discs were then thoroughly rinsed in Reverse Osmosis (RO) water and dried in a laminar flow cabinet. Ten discs, each from different leeks were used for each isolate and placed in a tray containing a moistened chux and paper towel. Each disc was treated with 0.5ml of Chloramphenicol to inhibit bacteria growth before a 6mm plug of a 10-14 day old *Fusarium* isolate grown on SNA was placed onto the centre of each disc and trays sealed in a plastic bag. The growth of *Fusarium* on discs was assessed 10-14 days after inoculation. Isolates that produced mycelial growth typical of *Fusarium* were re-tested using the seedling assay to confirm their pathogenicity.

Leek leaf assay

Leek leaves of unknown cultivar were sterilised by immersing for 3 minutes in a 4% hypochlorite solution, rinsed thoroughly and dried in a laminar flow cabinet. Leaves were placed in a tray containing a moistened chux and paper towel. Inoculum was prepared by removing conidia from infected leaves by shaking 5mm pieces of infected tissue in a 40ml solution of sterile water and Tween 20 for 10 seconds. Suspensions of 3.7×10^5 (*Stemphylium*) and 6.5×10^4 (*Alternaria*) spores/ml were then sprayed onto sterilised leek leaves and trays sealed in plastic bags for 7 days under a 12 hour cycle of UV light. After 7 days incubation, pathogenicity was confirmed when leaf spotting was observed similar to that seen in the field.

Results

Of the 355 *Fusarium* isolates tested for pathogenicity 67 (19%) were pathogenic causing foot rot on seedlings or produced pink mycelial growth on leek discs. These isolates were grouped according to morphological characteristics and representative isolates sent for identification.

Four species of *Fusarium* were identified and isolates lodged with the Australian Collection of Plant Pathogenic fungi as *Fusarium avenaceum*, *Fusarium oxysporum*, *Fusarium sambucinum* and *Fusarium culmorum*. The main pathogenic species were *F. avenaceum* and *F. oxysporum* being found in most states.

All 5 isolates of *Stemphylium* tested were pathogenic on leaf tissue. Two out of the 5 *Alternaria* isolates were pathogenic.

Bacteria

Materials and methods

Pathogenicity was tested using two isolates of *Pseudomonas syringae* pv. *porri*, recovered from leaf tissue in South Australia. Colonies from NA slopes were transferred into 100ml of nutrient broth and placed in an automatic stirrer for 48 hours at 25°C. Leek seed cv. Missile was germinated in 10cm tubs containing a sterile 50:50 perlite: vermiculite mix, watered and maintained in a growth room at 25°C for 2-4 weeks before seedlings were transferred to 5cm pots and grown for a further 3-4 weeks before inoculation.

A hypodermic syringe was used to inject 500µl of a bacterial suspension into 5 plants at soil level. Control plants were injected with RO water. Plants were then placed in a mist tent at 100% humidity for 7 days and then maintained in a greenhouse at 25°C. Plants were assessed 14 and 49 days after inoculation.

Results

All plants developed leaf spotting, curling and yellow leaf streaking 14 days after inoculation. Infection spread 5-10cm from the point of inoculation and symptoms were similar to that observed in the field. No control plants developed leaf spotting or streaking. Two plants showing leaf streaking and spotting were checked for bacterial streaming and isolated onto KB media. Bacterial streaming was observed and fluorescent colonies grew when streaked onto KB media, indicating the infection was caused by *Pseudomonas*. Representative isolates were lodged with the Australian Collection of Plant pathogenic bacteria as DAR 75555, DAR 75556 and DAR 75283.

Leek diseases in Australia

The four major diseases of leeks found during this survey were Fusarium foot rot, bacterial blight, leaf blight and purple blotch.

Foot rot caused primarily by *F. avenaceum* and *F. oxysporum* was observed in leek plantings in South Australia, Victoria and Western Australia throughout the 5-7 month growing season. Both species were recovered from basal plate rot and crown rot, no difference in species was found from the two symptoms. Severely diseased leeks were stunted and wilted. Plants could be pulled up easily in the field and a brown/yellow or pink crown rot observed at the base of the stem or as a pink rot of the basal plate. Symptoms were observed in seedlings before planting, 2-4 weeks after transplanting and in mature plants. *Fusarium* was also isolated from the roots and crowns of seedlings that were not showing symptoms.

Bacterial blight lesions and water soaked tissue were observed on the leaves of leek seedlings and mature plants in South Australia, Victoria and Western Australia. Symptoms appeared as brown leaf lesions surrounded by a narrow yellow halo, longitudinal water soaked tissue extending as a narrow strip from the leaf tip to the crown and brown longitudinal stripes on leek stems. Infected leaves were often curled, water soaked and light green in colour. Damage to seedlings was widespread resulting in seedling loss and poor establishment. The bacteria was identified as *Pseudomonas syringae* pv. *porri.*

Two fungi were found to cause leaf lesions and cosmetic damage on mature plants, namely leaf blight caused by *Stemphylium botryosum* and purple blotch caused by *Alternaria porri*.

Leaf blight was observed in mature leek plantings in South Australia, Victoria and Western Australia. Symptoms appeared as pale oval leaf lesions that turn brown after spore production causing widespread cosmetic damage.

Purple blotch was observed on two leek properties in Victoria and was found in Queensland. Symptoms appeared as oval purple lesions that darken with spore production. In severe infections blotching extended down the stem of the leek causing serious cosmetic damage and plant death.

Other diseases found on leeks in Australia were Pink root caused by *Pyrenochaeta terrestris*, Smudge caused by *Colletotrichum circinans*, Botrytis leaf spot caused by *Botrytis cinerea* and *Cladosporium* sp. (Table 1). These fungi have been isolated from plantings in South Australia during the survey but were not considered to cause significant problems.

Leek samples exhibiting yellow leaf streaking were collected from South Australia and Victoria during the survey and sent to virologists for identification. These tests confirmed the presence of Leek Yellow Stripe, Shallot Latent and Onion Yellow Dwarf viruses on leeks in Australia, but these viruses were not widespread.

Organisms collected during the survey found to cause damage to leeks in South Australia, Victoria, and Western Australia were onion maggot (*Delia antiqua*) and 3 species of parasitic nematodes, *Paratrichodorus* sp. (stubby root), *Pratylenchus* sp. (root lesion) and *Ditylenchus* sp. (stem and bulb). Onion maggot causes damage when the adult flies lay their eggs in the soil and hatching larva feed on plant tissue. Stubby root can cause considerable damage to plantings, stunting root growth and making it difficult for plants to take up sufficient nutrients and water.

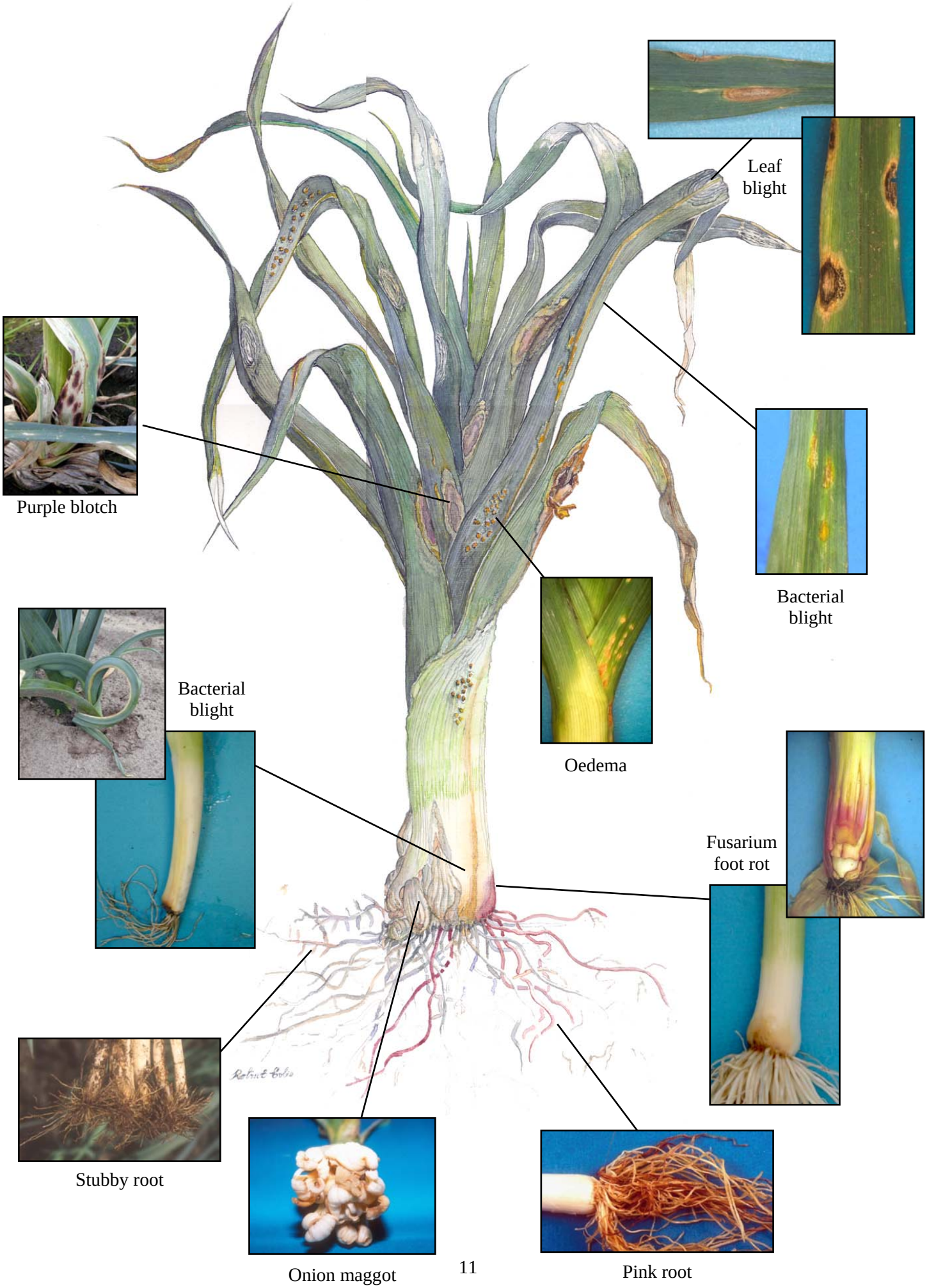
Oedema was found on leeks in plantings in South Australia and Victoria. It caused cosmetic damage to leeks and is triggered by environmental conditions of high soil moisture combined with cool nights and warm days.

Table 1. Pathogens and organisms found during 2000-2003 leek survey.

	South Australia	Victoria	Western Australia	Queensland
Fusarium	✓	✓	✓	-
Pseudomonas	✓	✓	✓	-
Stemphylium	✓	✓	✓	✓
Alternaria	-	✓	-	✓
Colletotrichum	✓	-	-	-
Botrytis	✓	-	-	-
Pyrenochaeta	✓	-	✓	-
Cladosporium	✓	-	-	-
Viruses	✓	✓	-	-
Onion maggot	✓	✓	✓	-
Nematodes	✓	✓	✓	-

N.B. More pathogens and organisms found in South Australia due to extensive surveying as properties were visited more frequently.

Picture 1. Diseases, disorders and organisms which affect leeks in Australia.



MANAGEMENT OF LEEK DISEASES

Fusarium foot rot

Introduction

Fusarium foot rot caused mainly by *F. avenaceum* and *F. oxysporum* was shown to be the main disease of leeks causing both a crown rot around the plant stem and a rot of only the basal plate. Studies were therefore carried out on the fungus and to investigate potential methods of control.

General materials and methods

Selective media: Three selective media were evaluated to determine the best media for the growth and maintenance of *Fusarium* isolates. Spezieller Nährstoffarmer Agar (SNA) (15), Carnation Leaf Agar (CLA), (15) and Dichloran Chloramphenicol Peptone Agar (DCPA) (15). SNA was the most efficient for the production of macro conidia and was used for growing inoculum.

Isolates: Isolates collected during the survey from various locations were used for laboratory, greenhouse and field experiments (Table 2).

Table 2. *Fusarium* isolates used for experimental work.

Isolate Number	Species	Location	Plant Part
18	*F. oxysporum*	Narine, SA	Crown
349	*F. avenaceum*	Langhorne Creek, SA	Roots
352	*F. avenaceum*	Langhorne Creek, SA	Roots
357	Pathogenic *Fusarium* sp.	Langhorne Creek, SA	Roots
361	Pathogenic *Fusarium* sp.	Murray Bridge, SA	Basal plate
362	*F. avenaceum*	Murray Bridge, SA	Basal plate
363	*F. avenaceum*	Murray Bridge, SA	Basal plate
409	*F. culmorum*	Langhorne Creek, SA	Crown
450	Pathogenic *Fusarium* sp.	Nairne, SA	Crown
451	*F. avenaceum*	Narine, SA	Crown
457	Pathogenic *Fusarium* sp.	Narine, SA	Crown
464	Pathogenic *Fusarium* sp.	Nairne, SA	Crown
468	Pathogenic *Fusarium* sp.	Devon Meadows, Vic	Crown
652	*F. sambucinum*	Devon Meadows, Vic	Crown
671	*F. culmorum*	Lenswood, SA	Seed
674	*F. avenaceum*	Lenswood, SA	Seed
677	*F. avenaceum*	Lenswood, SA	Seed
682	*F. avenaceum*	Lenswood, SA	Seed

Mycelial growth assessment: Fungal growth was measured as the mean of 2 radial dissects of the colonies and the percent inhibition determined relative to the growth of the control.

Detection and isolation of Fusarium: Leek crowns and roots were surface sterilised by soaking in 4% sodium hypochlorite solution for 3 minutes before rinsing thoroughly with RO water and dried in a

laminar flow cabinet. 2mm sections of leek tissue from the diseased margin were removed and plated on TWA, PDA and SNA. Plates were sealed with parafilm and incubated at 22^{o}C with a 12 hour photoperiod for 7-10 days.

Seed germination: Leek seed was surface sterilised in 70% ethanol before being dried in a laminar flow cabinet. Seeds were germinated in 10cm pots containing a sterilised 50:50 perlite: vermiculite mix, watered and maintained in a growth room at 25^{o}C. After 2 weeks seedlings were transferred into larger pots or speedling trays for experiments.

Inoculum: Fusarium isolates were grown on SNA for 7-10 days before 10ml of sterilised water was added to each agar plate and the *Fusarium* macro conidia removed from the agar into suspension with a sterile paintbrush. This suspension was collected and the process repeated. The inoculum suspension was filtered through gauze and adjusted to the required spore concentration. *Fusarium* inoculum was applied to the base of each plant.

Fungicide application: In greenhouse experiments fungicide/biologicals were applied to leek seedlings as drenches. Speedling trays were soaked with chemicals for 3 minutes to ensure trays were completely saturated. In field trials, chemicals were applied to seedlings using the same method prior to planting or by fungigation (post planting irrigation with a chemical solution). Where fungigation was used, chemicals were applied using a motorised backpack sprayer at rates of 300L/Ha.

Field trial locations: Field trials were undertaken at the Lenswood Research Centre located 30km east of Adelaide, SA in 2001, 2002 and 2004. Large scale field experiments were carried out in 2001 and 2002 on growers properties located at Nairne, Murray Bridge and Langhorne Creek located 50km east, 80km south east and 80km east of Adelaide respectively. Two field trials were set up in Victoria in 2004 located at Clyde and Cranbourne situated 40km and 45km south east of Melbourne respectively.

Leek disc assessment: Incidence and severity of *Fusarium* mycelial growth on leek discs was assessed after 14 days incubation. A 0-4 rating scale was used where 0 = no disease, 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% of the leek discs infected.

Greenhouse assessment: Dead and missing plants were recorded every 7-14 days. Seedlings with foot rot symptoms were isolated to determine whether *Fusarium* was the cause of seedling decline.

Field trial assessment: Leek plantings were assessed during the growing season for missing and dead plants. At harvest, leeks were removed from the ground, washed and assessed for the incidence and severity of foot rot. Foot rot was observed as either a crown rot on the leek stem or a basal plate rot. The two types of rot commonly occurred together with both species of *Fusarium* isolated from both symptoms with *F. avenaceum* the predominant species. The two rot symptoms were assessed together as foot rot infection where incidence of foot rot is the percent of leeks infected and foot rot severity is the percent of the leek area infected. Assessments were carried out using a 0-4 rating scale where 0= no disease, 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% of the leek infected.

Experimental design and statistical analysis: All trials were set up in a randomised block design unless otherwise specified. All data was analysed using the program "Statistix for Windows V8". In most instances the standard ANOVA was used on raw data unless otherwise specified.

Chemical and biological control: Fungicide and biological control agents were evaluated for their efficacy for the control of *Fusarium* (Table 3), selected either because they were known control *Fusarium* on other crops or were new chemistries. Fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks.

Table 3. Chemicals and biologicals evaluated for the control of Fusarium foot rot. The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Material	**Manufacturer**	**Active Ingredient**
Fungicide		
Bavistin ®	BASF	500g/L carbendazim
Bravo plus®	Syngenta	500g/L chlorothalonil + 10g/L cyprocazole
Cabrio®	BASF Aust Ltd	250g/L pyraclostrobin + 705g/L hydrocarbon liquid
Dithane®	Dow Agrosciences Aust	750g/Kg mancozeb
Filan®	BASF Aust Ltd	500g/Kg boscalid
Flint®	Bayer Cropscience	500g/Kg trifloxystrobin
Folicur®	Bayer Cropscience	430g/L tebuconazole
Maxim®	Syngenta	100g/L fludioxonil
Octave®	Bayer Cropscience	462g/L prochloraz
P-Pickel T ®	Crop Care Australasia	200g/L thiabendazole + 360g/Kg thiram
Ridomil Gold 480 EC ®	Syngenta	480g/L metalaxyl-M
Ridomil Gold MZ®	Syngenta	640g/Kg mancozeb + 40g/Kg metalaxyl
Rubigan®	Du Pont (Aust) Ltd	120g/L fenarimol
Switch®	Syngenta	375g/Kg cyprodinil + 250g/Kg fludioxonil
Tecto®	Syngenta	500g/L thiabendazole
Teldor®	Bayer Cropscience	500g/L fenhexamid
Biological		
Companion®	Growth Products Ltd	0.015% *Bacillus subtilis* (GB03)
Trichoflow®	Agrimm Technologies Limited	*Trichoderma* (minimum 100 million cfu's/g)
Trichogrow®	Agrimm Technologies Limited	*Trichoderma* (minimum 25 million cfu's/g)
Vinevax®	Agrimm Technologies Limited	*Trichoderma* (minimum 5 billion cfu's/g)

Laboratory experiments

In vitro

Objective: *To determine the effect of temperature on the growth of Fusarium and conidia formation.*

These studies were undertaken to determine the optimum temperature for the growth of *Fusarium*, to use for other experiments and to determine whether different isolates were effected by temperature.

Materials and methods

Three pathogenic *Fusarium* isolates (357, 457 and 464) and one *F. avenaceum* isolate (349) recovered from plantings in South Australia were used. A 5mm plug of agar taken from the outer edge of a 10 day old culture was plated onto petri plates containing TWA. Plates were incubated in the dark at 5, 10, 15, 20, 25, 30 and 35^{o}C and after 10 days the radial mycelial growth recorded on each of the 5 replicates for each temperature. The plates were then stored at room temperature (22^{o}C) for a further 7 days and assessed to determine if temperature inhibited the subsequent growth. At this time 5 mycelial plugs from each replicate were removed from the margin of each colony and macerated in 5ml of demineralised water and the number of macro conidia counted using an haemocytometer. This experiment was repeated using one pathogenic *Fusarium* isolate (357) and 4 *F. avenaceum* isolates (671, 674, 677 and 682) also collected from the Adelaide Hills.

Results and Discussion

The optimum temperature for the growth of all isolates was between 20 and 25^{o}C and all isolates of pathogenic *Fusarium* produced similar trends in growth. Temperatures of 30^{o}C or greater reduced the growth rate with no growth occurring at 35^{o}C (Figure 1 & 3). Mycelial growth at 30^{o}C was reduced to levels similar to that at 5^{o}C. When held at room temperature for a further 7 days all isolates continued to grow with temperature having no inhibitory effects.

No conidia developed on isolates grown at 5, 10, 15 or 35^{o}C and limited numbers were produced at 20 and 25^{o}C. Conidia production was favourable at 30^{o}C with 10 – 44 x 10^{5} macro spores/ml produced (Figure 2). No conidia developed in the second experiment.

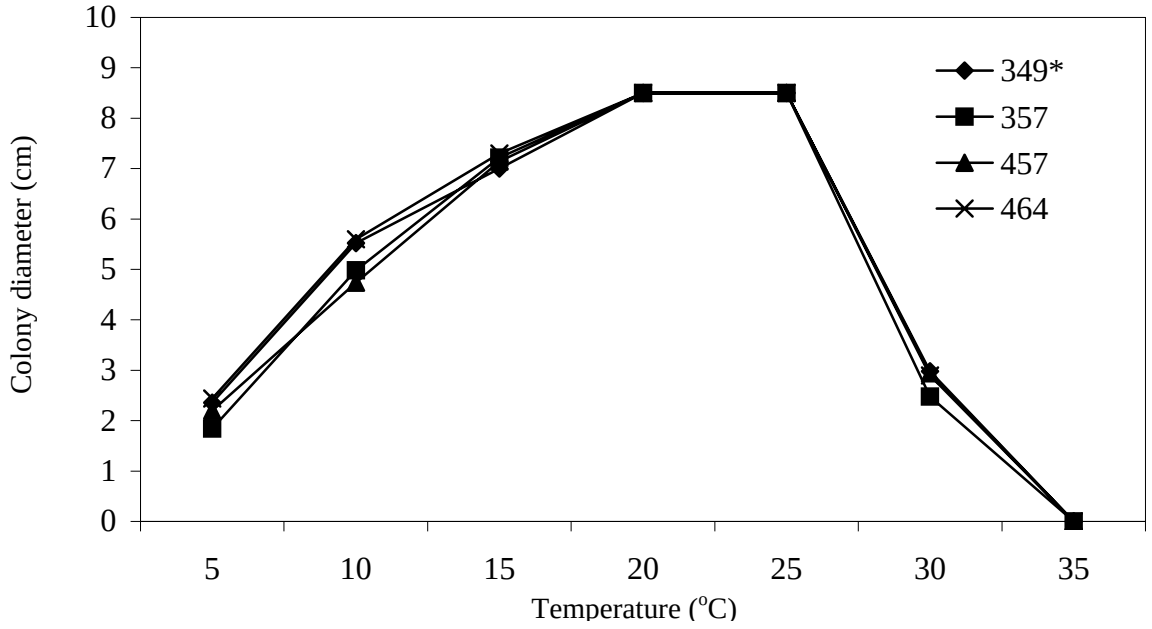


Figure 1. Effect of temperature on the mycelial growth of 1 *F. avenaceum** and 3 pathogenic *Fusarium* isolates.

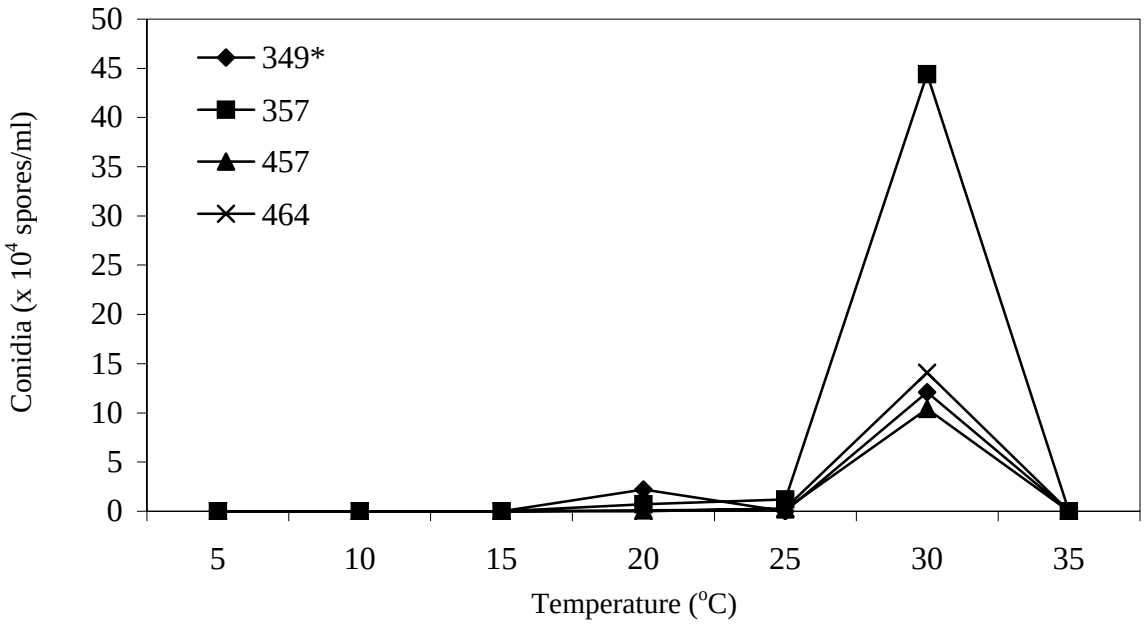


Figure 2. Effect of temperature on conidia formation of 1 *F. avenaceum** and 3 pathogenic *Fusarium* isolates.

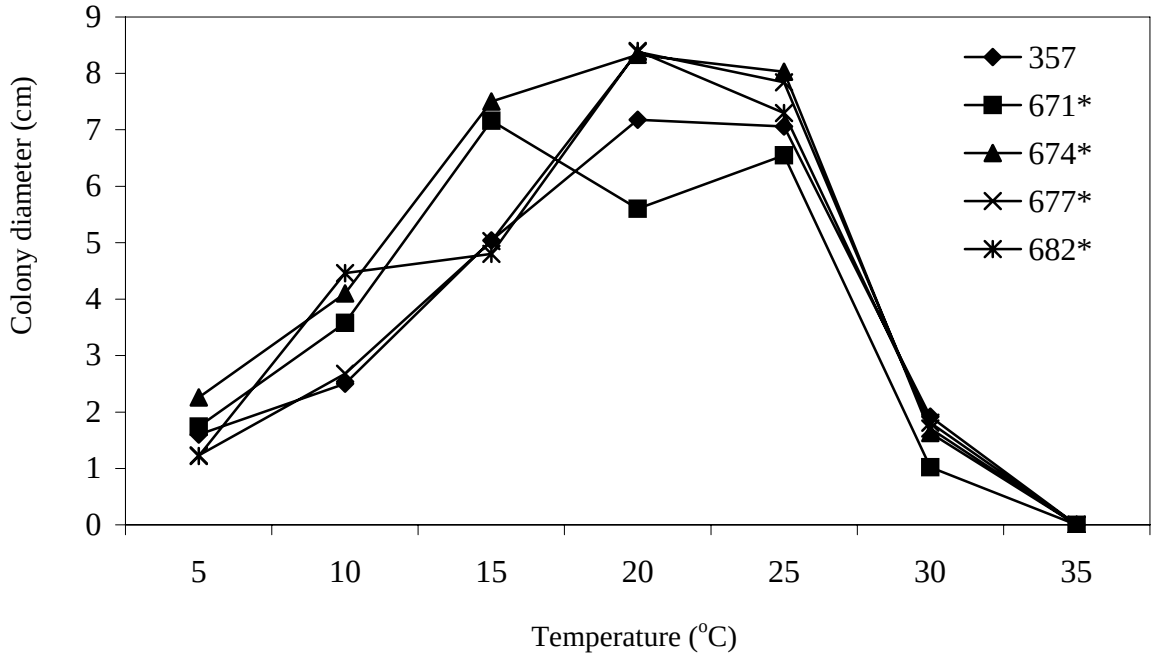


Figure 3. Effect of temperature on the mycelial growth of 4 *F. avenaceum** and 1 pathogenic *Fusarium* isolates.

Objective: *To evaluate the sensitivity of Fusarium to various fungicides.*

These studies were carried out to screen fungicides for potential use as soil drench treatments for the control of *Fusarium*. Fungicides and biological control agents were evaluated by determining their inhibitory effect on the mycelial growth of *Fusarium* using amended agar and leek discs. The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Materials and methods

Experiment 1

Three isolates of *F. avenaceum* (349, 362 and 451) and 2 pathogenic *Fusarium* isolates (361 and 450) were used to evaluate the inhibitory effect of fungicides *in vitro*. PDA was amended with either chlorothalonil + cyprocazole, fludioxonil, carbendazim, fenhexamid, tebuconazole, prochloraz or thiabendazole + thiram at 0.01, 0.1, 1, 5, or 10ppm active ingredient. Fungicide suspensions were prepared as stock solutions from formulated products and added to agar just prior to pouring the plates at 55-60^{o}C. The plates were dried in a laminar flow cabinet for 30 minutes before a 5mm plug of agar taken from the outer edge of an actively growing pathogenic *Fusarium* isolate was placed in the centre of 5 agar plates of each concentration. The plates were sealed with parafilm and incubated at 25^{o}C for 5 days and the radial diameter of mycelial growth measured and percent inhibition determined as previously mentioned.

Experiment 2

The most effective fungicides from experiment 1 were also evaluated on an isolate of *F. oxysporum* (18). This experiment was undertaken using the same methods as experiment 1 except PDA was amended with either carbendazim or prochloraz at 0.01, 0.1, 1, 5, or 10ppm active ingredient.

Results and discussion

All the fungicides evaluated inhibited the mycelial growth of *F. avenaceum* and pathogenic isolates. Prochloraz and fludioxonil were the most inhibitory with 100% inhibition at 1ppm. Low concentrations of prochloraz were effective with 50% inhibition of fungal growth at 0.01ppm. Carbendazim, thiabendazole + thiram and tebuconazole were also effective with complete inhibition at 5ppm. Fenarimol and chlorothalonil + cyprocazole did have some effect on reducing the growth of *Fusarium* however complete inhibition was not achieved at any of the concentrations tested (Figures 4 – 10).

The effect of prochloraz on the growth of *F. oxysporum* was similar to that achieved for *F. avenaceum* with complete inhibition at 0.1ppm. Carbendazim was less effective than prochloraz with complete inhibition of mycelial growth at 1ppm (Figure 11).

These preliminary *in vitro* studies showed prochloraz to be the most effective treatment for the control of both *F. avenaceum* and *F. oxysporum*.

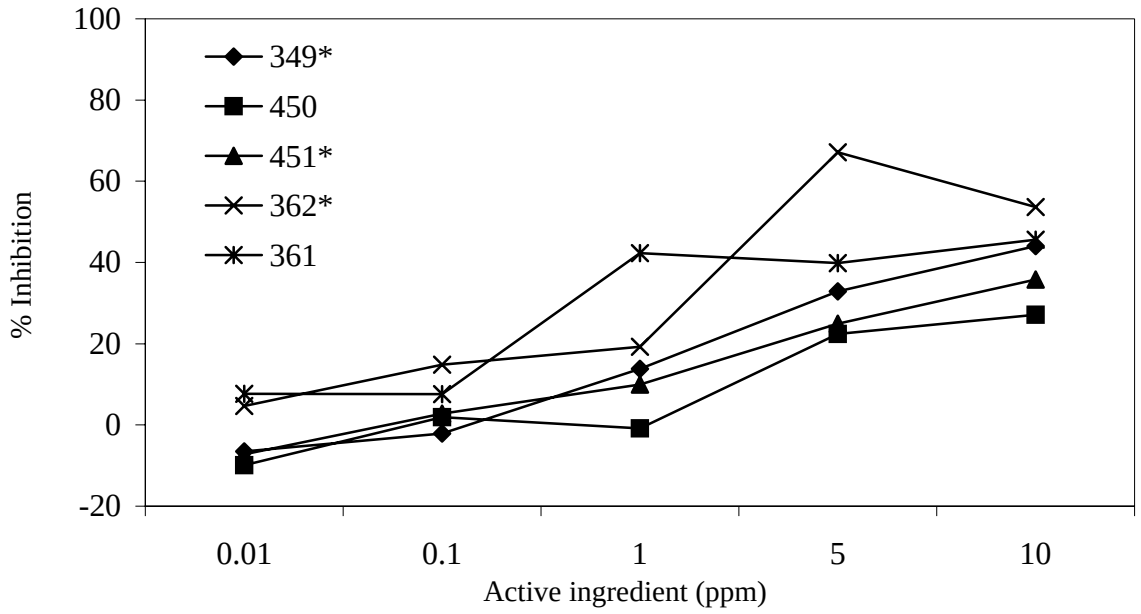


Figure 4. Effect of different concentrations of chlorothalonil + cyprocazole on the mycelial growth of 3 *F. avenaceum** and 2 pathogenic *Fusarium* isolates.

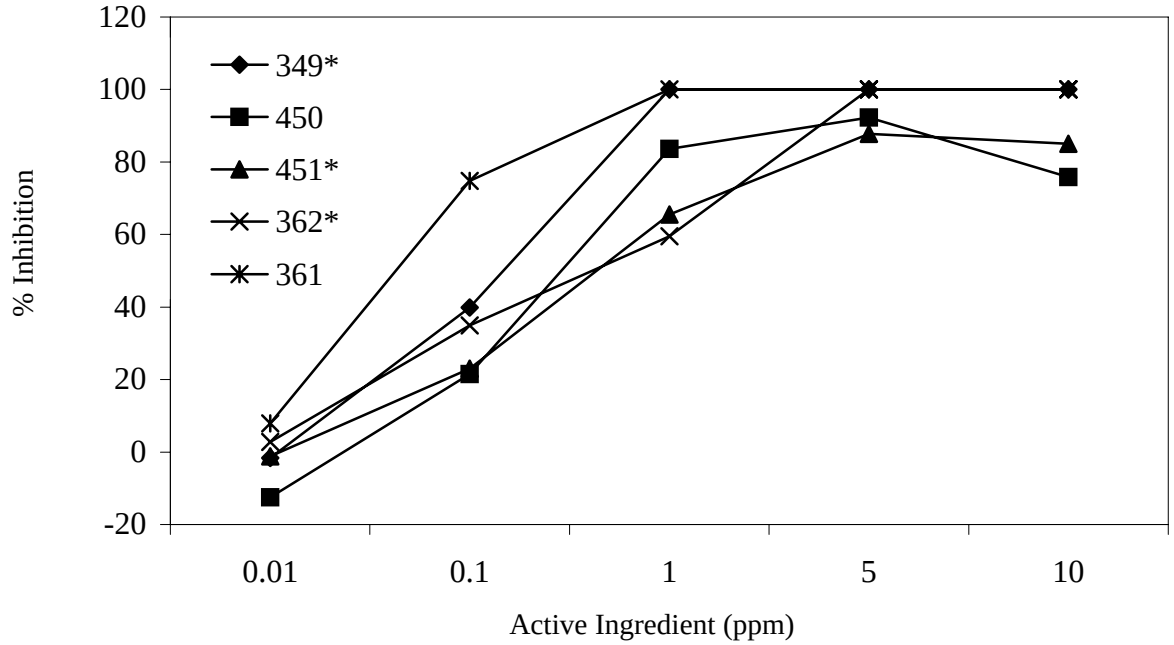


Figure 5. Effect of different concentrations of fludioxonil on the mycelial growth of 3 *F. avenaceum** and 2 pathogenic *Fusarium* isolates.

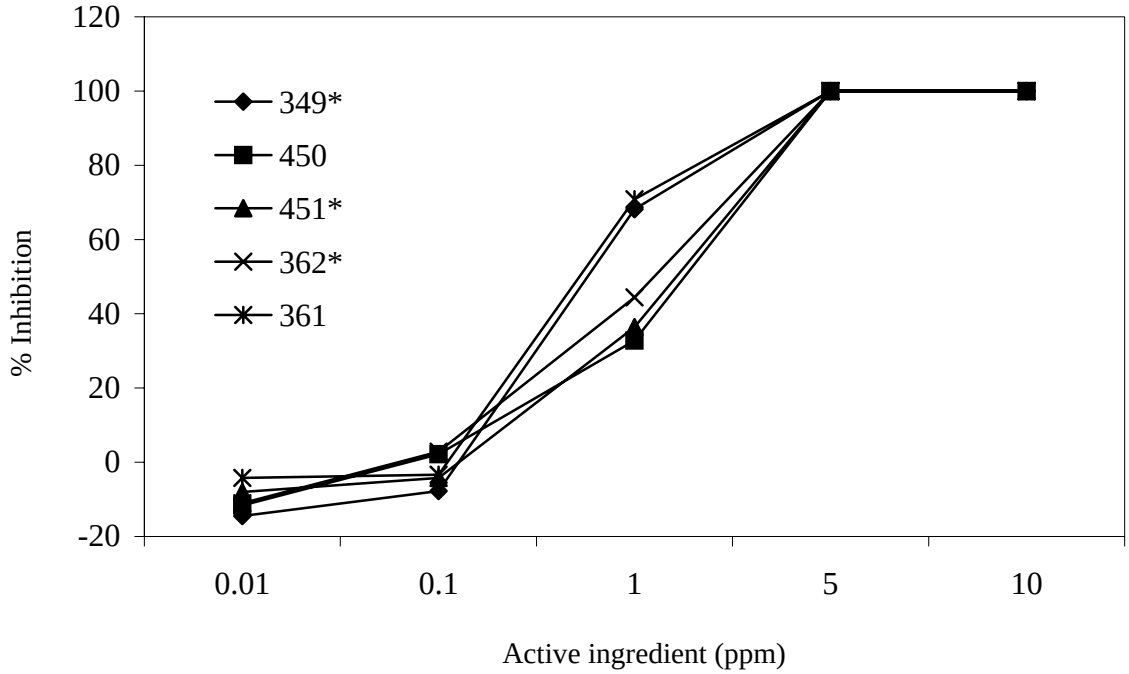


Figure 6. Effect of different concentrations of carbendazim on the mycelial growth of 3 *F. avenaceum** and 2 pathogenic *Fusarium* isolates.

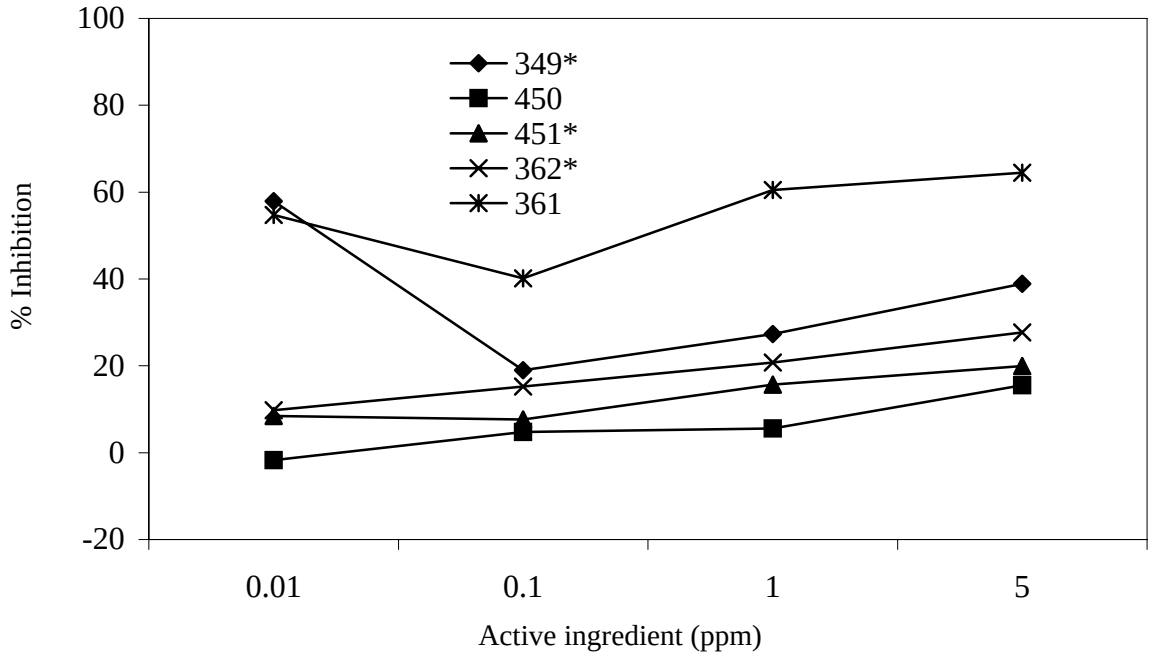


Figure 7. Effect of different concentrations of fenarimol on the mycelial growth of 3 *F. avenaceum** and 2 pathogenic *Fusarium* isolates.

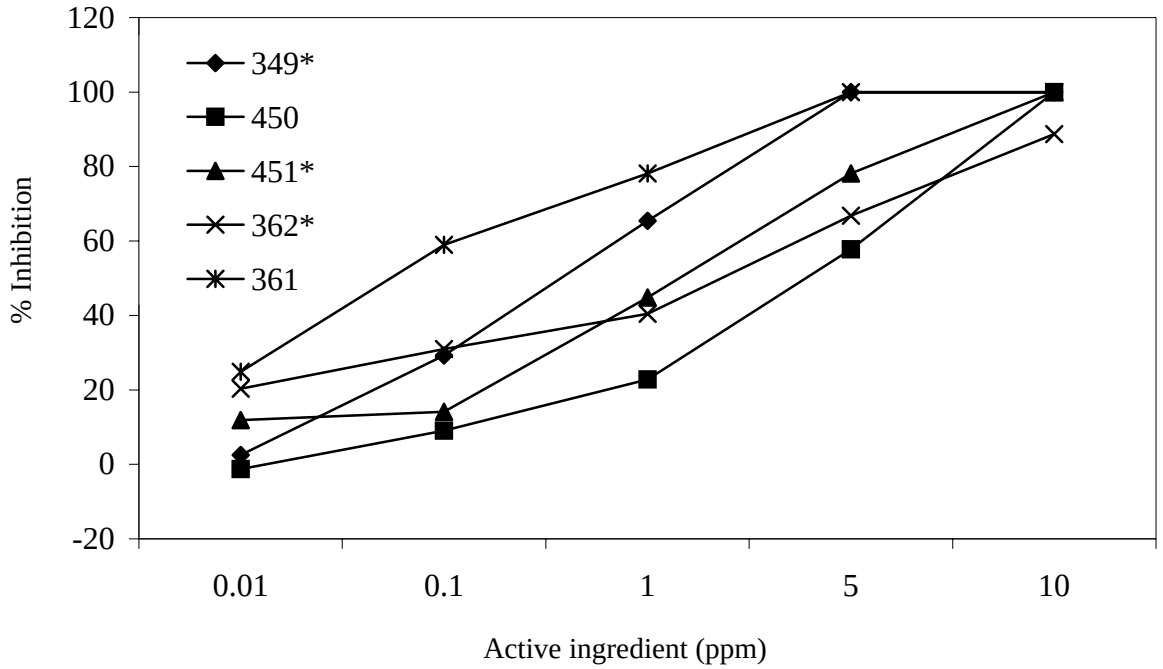


Figure 8. Effect of different concentrations of tebuconazole on the mycelial growth of 3 *F. avenaceum** and 2 pathogenic *Fusarium* isolates.

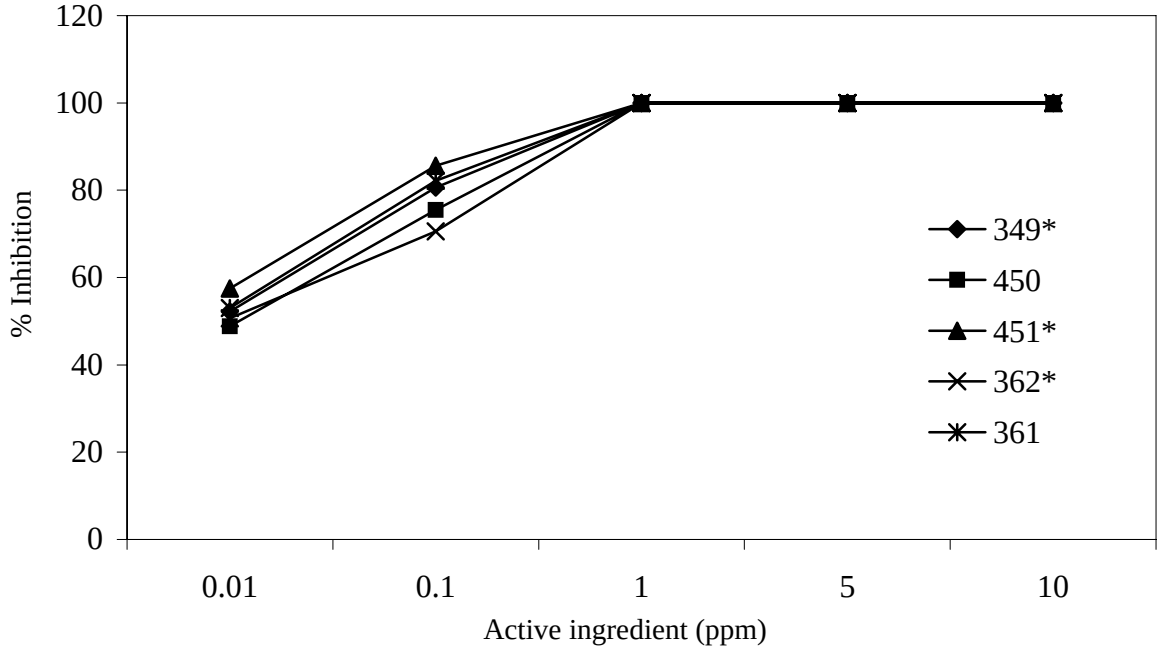


Figure 9. Effect of different concentrations of prochloraz on the mycelial growth of 3 *F. avenaceum** and 2 pathogenic *Fusarium* isolates.

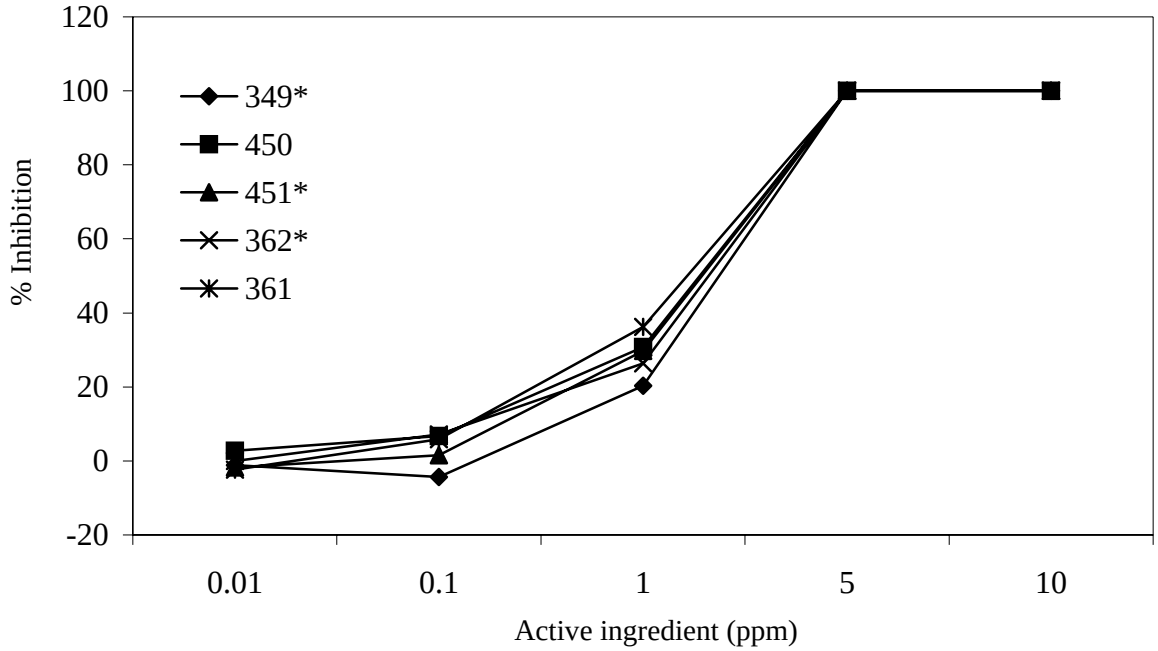


Figure 10. Effect of different concentrations of thiabendazole + thiram on the mycelial growth of 3 *F. avenaceum** and 2 pathogenic *Fusarium* isolates.

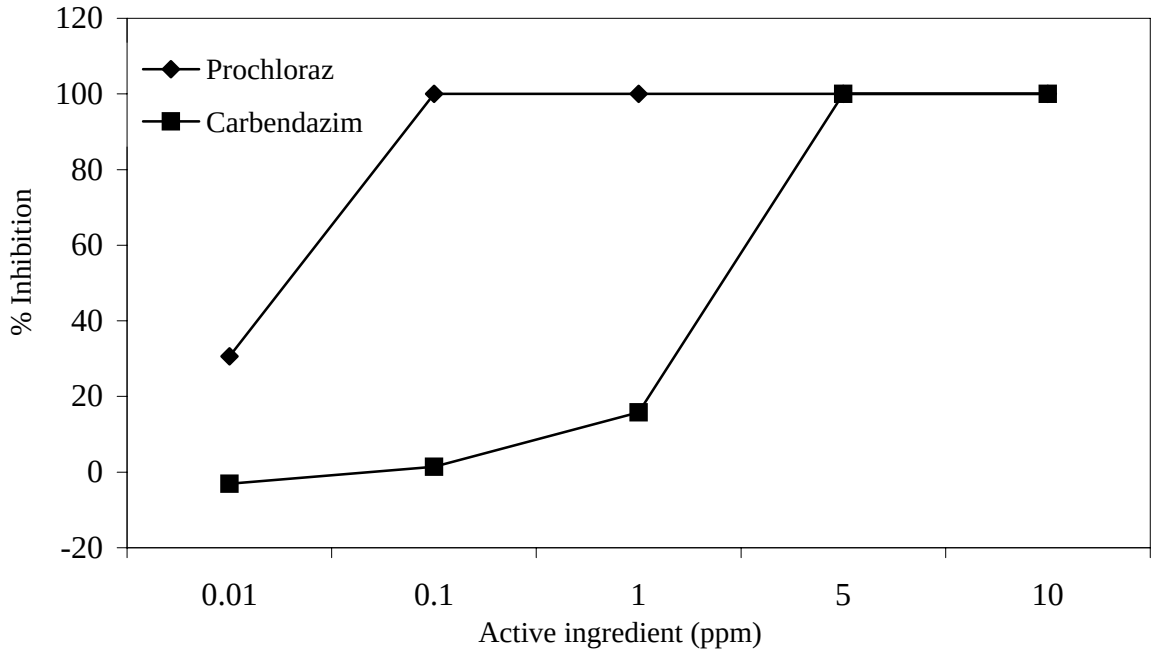


Figure 11. Effect of different concentrations of carbendazim and prochloraz on the mycelial growth of *F. oxysporum*.

Leek disc experiments

Objective: *To evaluate the potential of 3 fungicides and 1 biological for the control of Fusarium.*

Materials and methods

Four experiments were undertaken to test the sensitivity of *F. sambucinum* (652) to 13 fungicides and 1 biological. Leek discs approximately 1cm thick were surface sterilised as previously described. Five discs for each treatment were placed in 200ml of a fungicide or biological solution for 3 minutes before being placed on trays containing a moistened chux and paper towel. All treatments were applied at 1000ppm active ingredient and are listed in Table 4. Each disc was inoculated by placing a 5mm mycelial plug of a 10 day old isolate of *F. sambucinum* grown on SNA in the centre of each disc and trays sealed in plastic bags. Trays were incubated for 14 days at room temperature and the incidence and severity of *F. sambucinum* infection assessed as previously described.

Table 4. Fungicides and biologicals tested for *F. sambucinum* control. The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Fungicide / biological	Active Ingredient	Experiment
Bavistin®	500g/L carbendazim	1, 2 & 3
Octave®	462g/L prochloraz	1, 2 & 3
P-Pickel T®	200g/L thiabendazole + 360g/Kg thiram	1 & 2
Companion®	0.015% *Bacillus subtilis*	1
Folicur®	430g/L tebuconazole	2 & 3
Maxim®	100g/L fludioxonil	2 & 3
Rubigan®	120g/L fenarimol	2
Ridomil Gold 480 EC®	480g/L metalaxyl-M	2
Ridomil Gold MZ®	640g/Kg mancozeb + 40g/Kg metalaxyl	2
Bravo Plus®	500g/L chlorothalonil + 10g/L cyprocazole	2
Teldor®	500g/L fenhexamid	3
Switch®	375g/Kg cyprodinil + 250g/Kg fludioxonil	3
Flint®	500g/Kg trifloxystrobin	3
Cabrio®	250g/L pyraclostrobin	4
Filan®	500g/Kg boscalid	4

Results and discussion

Experiment 1

Fusarium sambucinum grew and sporulated extensively on all untreated discs and on 100% of discs treated with the biological *Bacillus subtilis*. The three fungicide treatments completely inhibited the development of *F. sambucinum* with no growth on discs treated with either prochloraz, thiabendazole + thiram and carbendazim (Table 5).

Experiment 2

As in experiment 1, prochloraz, thiabendazole + thiram and carbendazim all completely inhibited the growth of *F. sambucinum* on leek discs, as did fludioxonil and tebuconazole. Fenarimol, mancozeb + metalaxyl, metalaxyl-M and chlorothalonil + cyprocazole were ineffective in controlling *F. sambucinum* with 100% of leek discs infected after 14 days (Table 5).

Experiment 3

Carbendazim and prochloraz again completely inhibited the growth of *F. sambucinum* on leek discs. Tebuconazole and fludioxonil were inhibitory but not as effective as in experiment 2 with 50 and 10% infection respectively. Fenhexamid and trifloxystrobin were not effective infecting 100% of leek discs while 10% of leek discs were infected when treated with cyprodinil + fludioxonil (Table 5).

Experiment 4

100% of leek discs treated with boscalid were infected with *F. sambucinum*. Pyraclostrobin provided some control with 20% of leek discs infected (Table 5).

The results of these experiments show that the most effective fungicides for inhibiting the growth of *F. sambucinum* are prochloraz, carbendazim and thiabendazole + thiram, with fludioxonil, tebuconazole, pyraclostrobin and cyprodinil + fludioxonil providing some control.

Table 5. Incidence and severity of *F. sambucinum* on leek discs treated with fungicides and biological control agents.

Treatment	Incidence				Severity (mean rating 0-4)			
	Expt 1	Expt 2	Expt 3	Expt 4	Expt 1	Expt 2	Expt 3	Expt 4
Carbendazim	0	0	0	-	0	0	0	-
Prochloraz	0	0	0	-	0	0	0	-
Thiabendazole + thiram	0	0	-	-	0	0	-	-
Bacillus subtilis	100	-	-	-	4	-	-	-
Tebuconazole	-	0	50	-	-	0	0.5	-
Fludioxonil	-	0	10	-	-	0	0.2	-
Fenarimol	-	100	-	-	-	3.6	-	-
Metalaxyl-M	-	100	-	-	-	4	-	-
Mancozeb + metalaxyl	-	100	-	-	-	4	-	-
Chlorothalonil + cyprocazole	-	100	-	-	-	4	-	-
Fenhexamid	-	-	100	-	-	-	4	-
Cyprodinil + fludioxonil	-	-	10	-	-	-	0.1	-
Trifloxystrobin	-	-	100	-	-	-	1.7	-
Pyraclostrobin	-	-	-	20	-	-	-	0.5
Boscalid	-	-	-	100	-	-	-	2.9
Positive control	100	100	70	100	4	4	1.5	2.9
Negative control	0	0	0	0	0	0	0	0
LSD (P=0.05)	-	-	25	26.5	-	0.34	0.58	-

Greenhouse experiments

A number of experiments were undertaken to determine the susceptibility of leek seedlings to Fusarium foot rot and to evaluate fungicide and biological drenches for control. The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Objective: *To determine the susceptibility of different leek varieties to Fusarium.*

Materials and methods

Leek seed cv. Missile, Harpoon, Nova and Admiral were germinated as previously described. After 2 weeks seedlings were planted into 5cm pots containing UC soil. Inoculum was prepared by growing 2 *F. avenaceum* isolates (349 and 362) and 1 pathogenic *Fusarium* isolate (361) on SNA for 14 days and macerating each plate with 30ml of RO water. These three isolates of *Fusarium* were tested on each variety of leek by applying 1.5ml of inoculum to the base of each plant. A SNA plate containing no fungi was also macerated and applied to control plants. Pots were lightly watered and maintained in a greenhouse at 25^{o}C and assessed for 12-14 weeks.

Results and discussion

Three weeks after inoculation seedlings were either killed or severely stunted as a result of foot rot. Symptomatic plants were removed from the pots and *F. avenaceum* re-isolated from the diseased tissue. The leek variety Harpoon was most susceptible to all three isolates of pathogenic *Fusarium* with 80% of plants dying compared to the varieties Nova, Missile and Admiral where 67% of plants died (Table 6).

Table 6. Percent of missing leeks artificially inoculated with *Fusarium.*

Variety	Dead plants (%)
Missile	67
Harpoon	80
Nova	67
Admiral	67
LSD (P=0.05)	ns

Where: ns = no significant differences detected between treatments.

Objective: *To determine the influence of seedling age and root damage on the susceptibility to Fusarium infection.*

Materials and methods

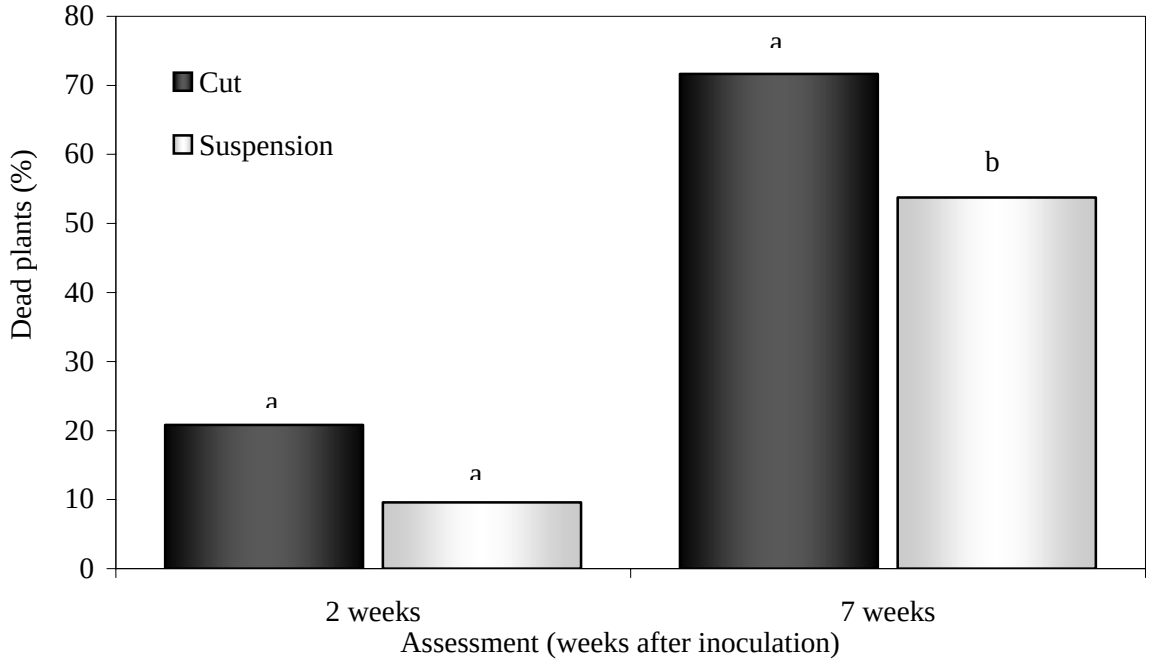
Leek seed cv. Amundo was germinated as previously described. After 2 weeks seedlings were transferred into speedling trays and maintained in the greenhouse at 26^{o}C. Seedlings at 2, 4, 6 and 8 weeks of age were inoculated with 2 *F. avenaceum* isolates (349 and 451), 1 isolate of *F. culmorum* (409) or a pathogenic *Fusarium* isolate (468) using two different inoculation methods. Seedlings were inoculated by either applying 1ml of a conidia suspension containing 10^6 macrospores/ml at the base of each plant or removing seedlings from trays, cutting the roots of seedlings to 2cm lengths and soaking in a conidia suspension containing 10^6 macrospores/ml for 3 minutes before replanting. Seedlings were assessed weekly for the number of dead and dying plants.

Results and discussion

Inoculating leek seedlings by cutting the roots and dipping into an inoculum suspension increased the incidence of dead plants after 2 weeks with 20% of seedlings missing compared to 9.5% in the suspension inoculation. Seven weeks after inoculation significantly more plants were missing from the cut root than the suspension inoculation method where 71% and 53% of seedlings were missing respectively (Figure 12).

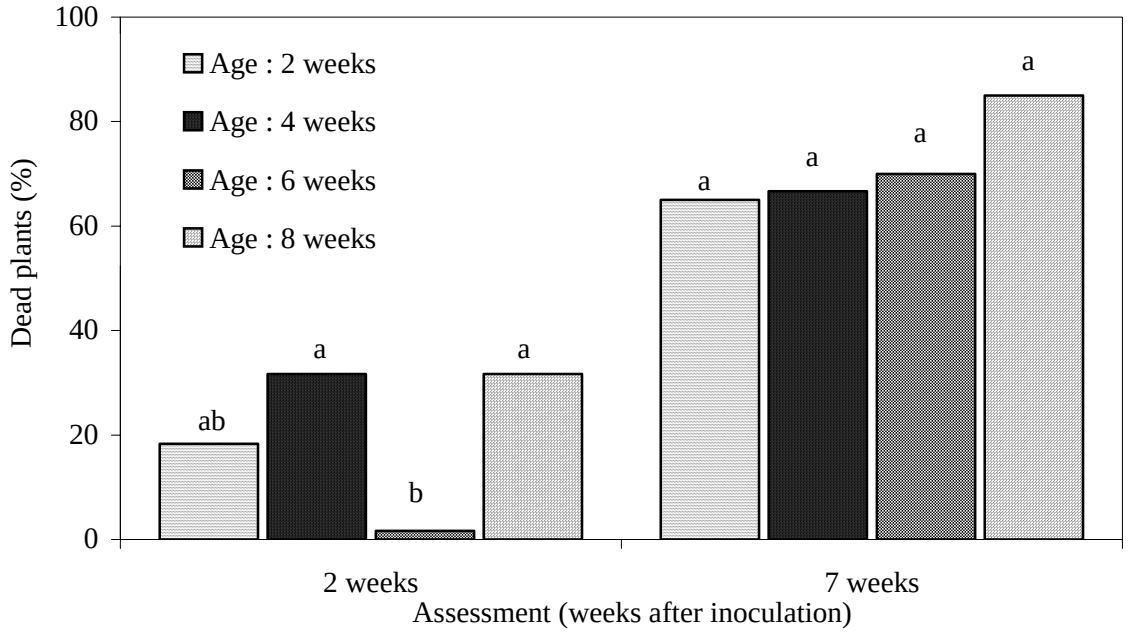
No differences were detected when seedlings were inoculated at either 2, 4, 6 or 8 weeks of age by either inoculation method except at 2 weeks in the cut root method, where only 1.6% of leeks were dead in the 6 week old seedlings compared to 31% in the 4 and 8 week old seedlings. Even though this difference is significant, after a further 5 weeks the incidence of dead plants was between 65-80% at all ages (Figure 13 & 14).

These results show that seedlings at 2, 4, 6, and 8 weeks of age are all susceptible to *F. avenaceum* and *F. culmorum* infection. It also indicates that damage to seedling roots (for example by nematodes) can increase the incidence of dead plants.



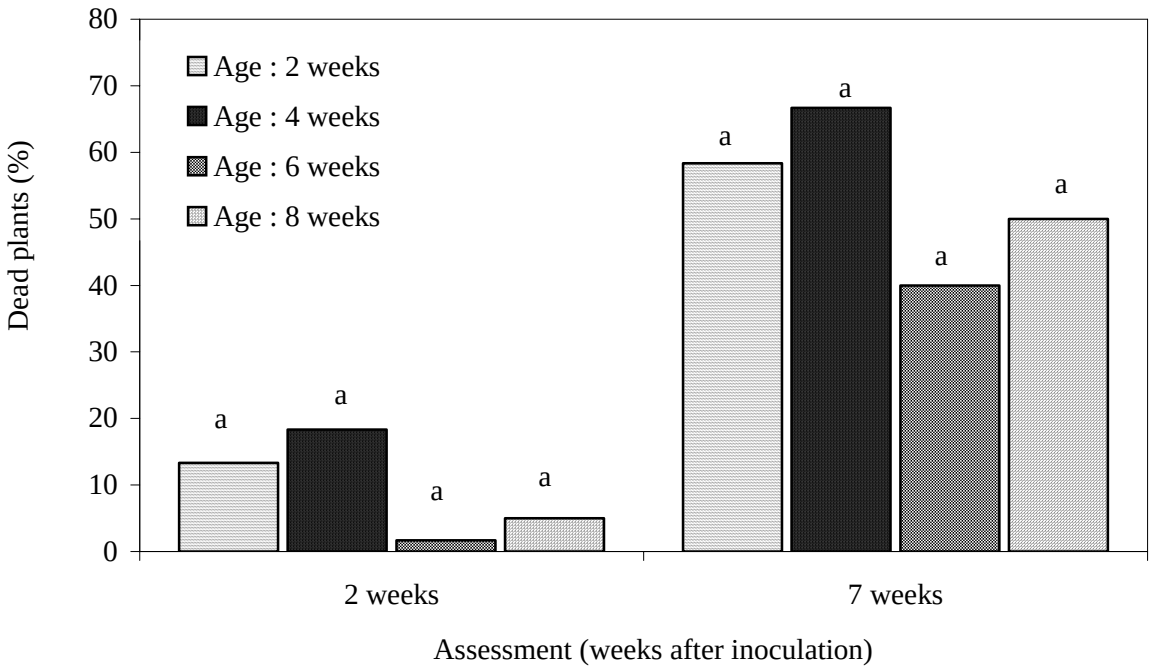
N.B. Treatments with the same letter are not significantly different from one another (P=0.05).

Figure 12. Incidence of dead plants after inoculating 2, 4, 6, or 8 week old seedlings with *F. avenaceum* or *F. culmorum* by either adding a spore suspension to the soil or cutting the roots and dipping in a spore suspension.



NB. Treatments with the same letter are not significantly different from one another (P=0.05).

Figure 13. Incidence of dead plants 2 and 7 weeks after inoculating 2, 4, 6, or 8 week old seedlings with *F. avenaceum* or *F. culmorum* by dipping the cut roots of seedlings in an inoculum suspension for 3 minutes and re planting.



NB. Treatments with the same letter are not significantly different from one another (P=0.05).

Figure 14. Incidence of dead plants 2 and 7 weeks after inoculating 2, 4, 6, or 8 week old seedlings with *F. avenaceum* or *F. culmorum* by applying a spore suspension to the base of the plant.

Objective: *To evaluate fungicide drenches for the control of Fusarium infection in seedlings.*

Materials and methods

Leek seedlings in all 3 drench experiments were inoculated with a combination of *F. avenaceum* isolates (349, 362, 451, 674, 677 and 682). The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Experiment 1

This experiment evaluated fungicide drenches applied either 2 or 7 days after *Fusarium* inoculation. Leek seed cv. Amundo was germinated as previously described and seedlings planted into speedling trays after two weeks. After 8 weeks growth, the seedlings were inoculated by applying a suspension containing 3.6×10^5 macrospores/ml to the base of each plant. Each tray contained 36 inoculated plants and was treated with fungicides or water either 2 or 7 days after inoculation. Fungicide solutions of either carbendazim (2ml/L), prochloraz (2.16g/L), thiabendazole (2ml/L), fludioxonil (10ml/L) or tebuconazole (2ml/L) were applied as a drench by soaking speedling trays in 2 litres of chemical solution and untreated controls in 2 litres of water for 3 minutes. Seedlings were assessed every 2 weeks for dead and missing plants.

Experiment 2

This experiment was undertaken to determine the effect of fungicide or biological drenches applied either 2 or 7 days after infection reduce the number of dead leek seedlings and whether inoculum concentration had an effect on disease incidence. This experiment followed the same method as in experiment 1 however inoculum concentrations of either 10^2, 10^4 or 10^6 macrospores/ml were added to the base of each plant. Each tray contained 36 inoculated plants and was treated with fungicides, a biological or water 2 or 7 days after inoculation. Fungicide or biological solutions of either carbendazim (2ml/L), prochloraz (2.16g/L), thiabendazole (2.77ml/L) or Trichoderma (Vinevax) (10g/L) were applied as drenches with untreated trays drenched in water. Seedlings were assessed weekly for dead and missing plants for 12-14 weeks.

Experiment 3

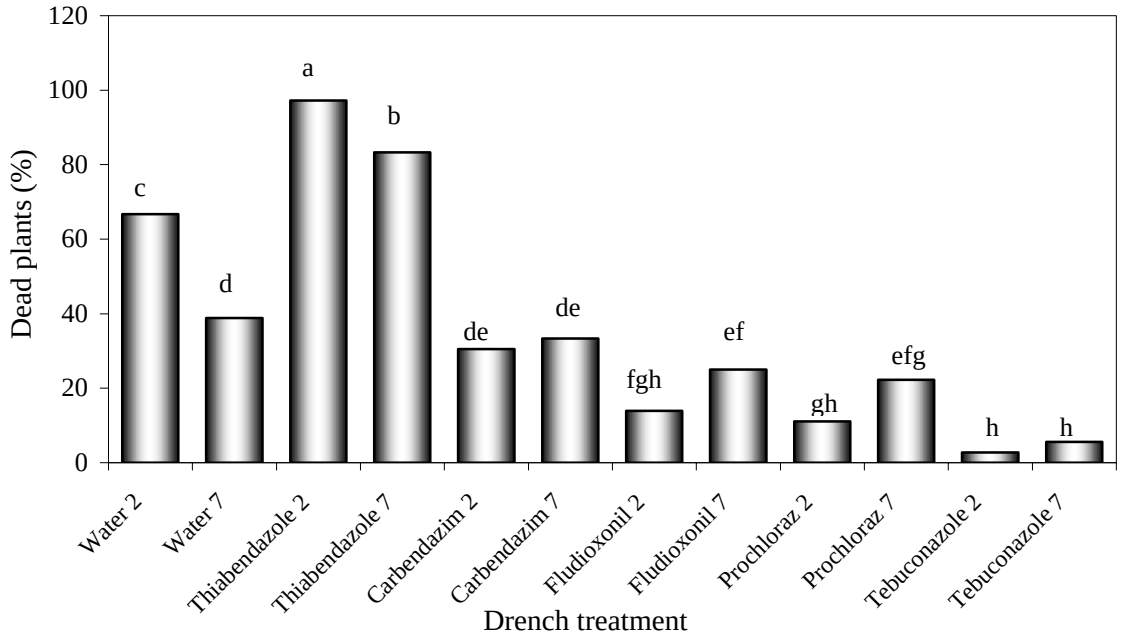
The third experiment evaluated fungicides applied as a drench to seedlings at 2 different rates and its effect on reducing seedling death. Seedlings were inoculated by applying a suspension containing 2.2×10^5 *Fusarium* macrospores/ml to the base of each plant. Each tray contained 36 inoculated plants and was treated with fungicide or water 7 days after inoculation. Fungicide solutions of either carbendazim (1 or 2ml/L), prochloraz (2 or 3g/L) or thiabendazole (1 or 2ml/L) were applied as a drench by soaking trays in a 2 litre suspension of chemical with untreated controls drenched in water. Seedlings were assessed for dead and missing plants every 2-3 weeks for 12 weeks.

Results and discussion

Experiment 1

Applying drench treatments to leek seedlings either 2 or 7 days after inoculation reduced the number of dead plants 12 weeks later. Significantly less dead plants were found in the fludioxonil, prochloraz and tebuconazole treatments compared to the water treated. Although no significant differences were detected, chemical applications at 2 days reduced the number of dead plants compared to the 7 day treatment. The most effective chemical treatment was tebuconazole applied at 2 and 7 days where 2.7 and 5.5% of plants were missing respectively. Fludioxonil and prochloraz treatments were also effective in reducing the number of dead leeks with 11 and 13% of leeks missing respectively when applied 2 days after inoculation. Carbendazim applied at 2 and 7 days reduced the number of dead plants but was not as effective as that seen in the treatments previously mentioned (Figure 15).

The number of leek seedlings missing in thiabendazole treatments was higher than the untreated at both application timings with 97 and 83% of leek seedlings missing at 2 and 7 days respectively. This suggests that the rates of thiabendazole used in this experiment were phytotoxic as leek seedlings reacted to the chemical treatment. Declining seedlings were removed from all treatments and roots and crown tissue re-isolated. No *F. avenaceum* was found in the thiabendazole treatments while *F. avenaceum* was recovered from the other treatments and the untreated controls.



NB. Treatments with the same letter are not significantly different from one another (P=0.05).
Where 2 = drench applied 2 days after inoculation and 7 = drench applied 7 days after inoculation.

Figure 15. Incidence of dead leek seedlings drenched in fungicide 2 or 7 days after inoculating with *F. avenaceum*.

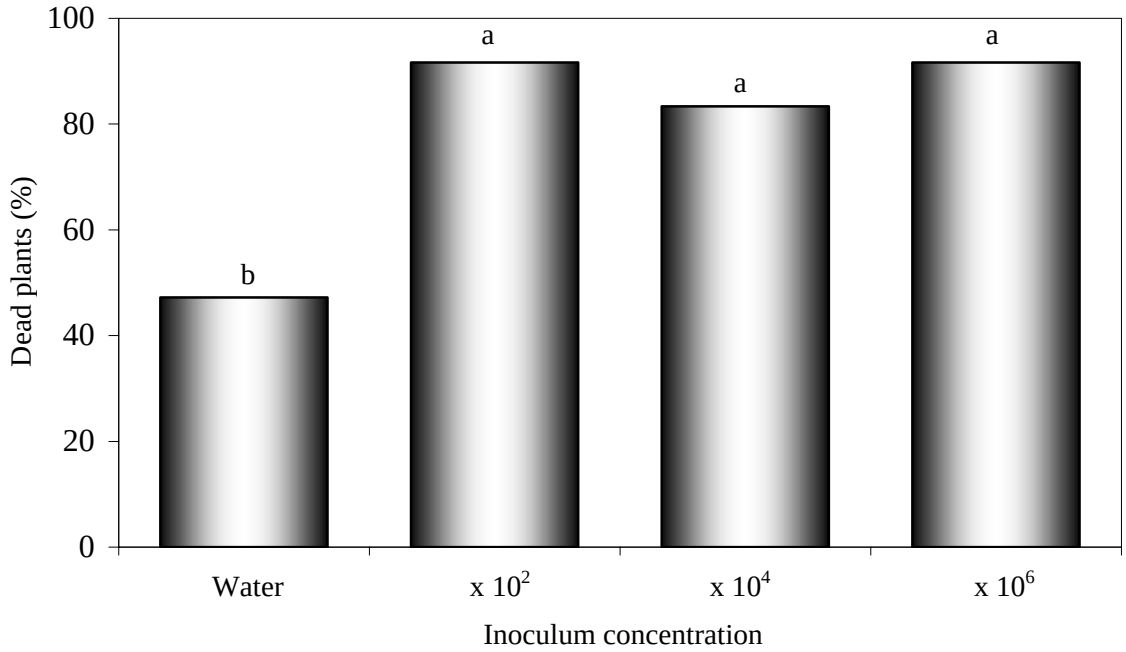
Experiment 2

Inoculating leek seedlings with different concentrations of *F. avenaceum* had no effect on the number of dead plants at the final assessment. All untreated plants inoculated with the 3 inoculum concentrations had significantly higher numbers of dead plants than those in the un-inoculated control. No significant difference between inoculum concentrations were detected (Figure 16).

Drench treatments of prochloraz and carbendazim were the most effective in reducing the number of dead plants (Figure 17). No plants were missing when seedlings were inoculated at 10^2 spores/ml and treated with prochloraz either 2 or 7 days later. The most effective carbendazim treatment was applied 7 days after being inoculated with the highest concentration of spores with no plant death recorded.

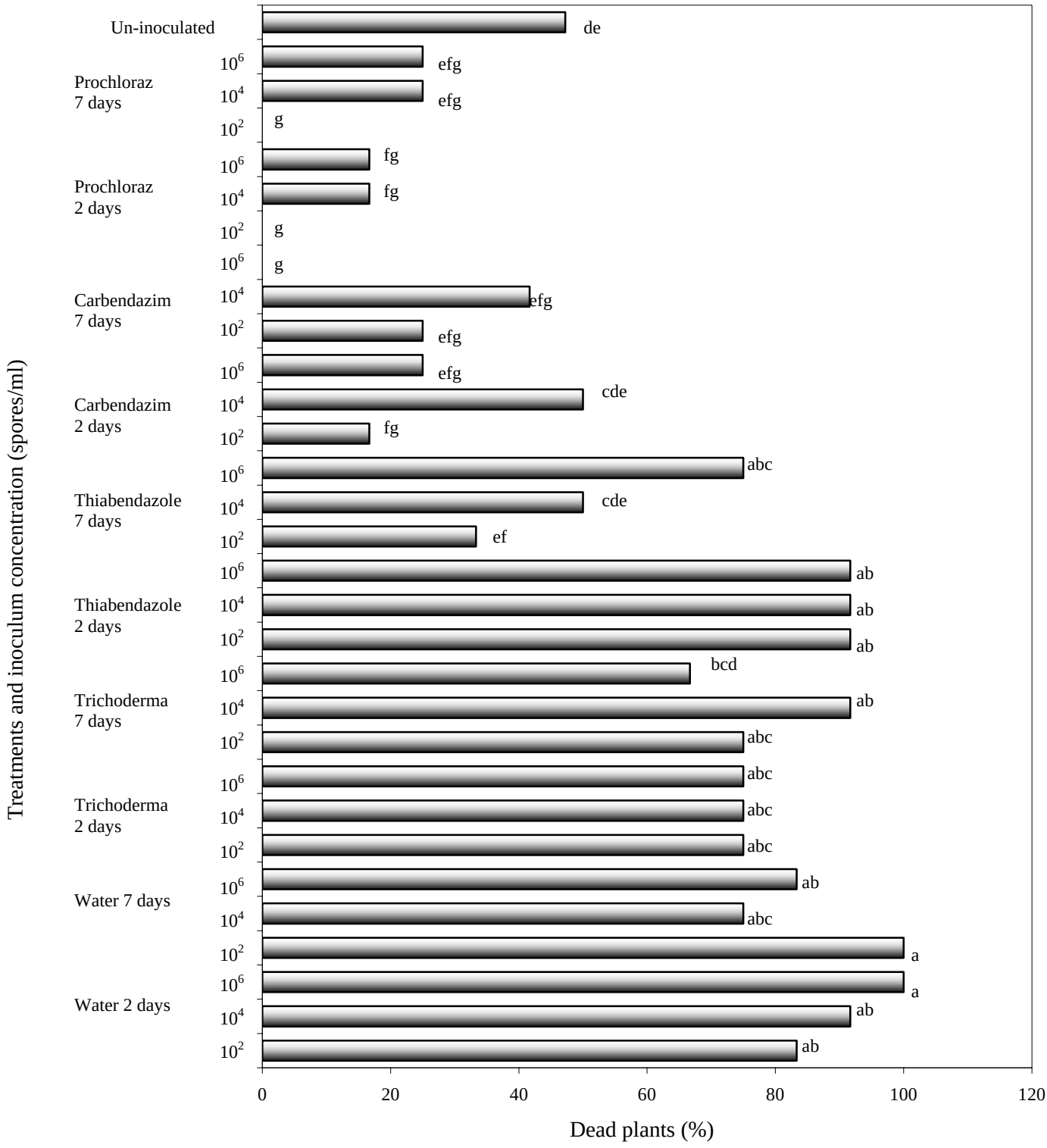
Thiabendazole applied 2 days after infection had little effect in reducing the number of dead plants with 91% of leek seedlings dead after 14 weeks. When applied 7 days after infection thiabendazole significantly reduced the level of dead plants in the lowest inoculum concentration with 33% of plants missing compared to 100% in the untreated (Figure 17).

Trichoderma treatments had little effect in reducing numbers of dead plants with the incidence in all treatments over 75%.



NB. Treatments with the same letter are not significantly different from one another (P=0.05).

Figure 16. Experiment 2. Incidence of dead plants inoculated with 3 different concentrations of *F. avenaceum.*



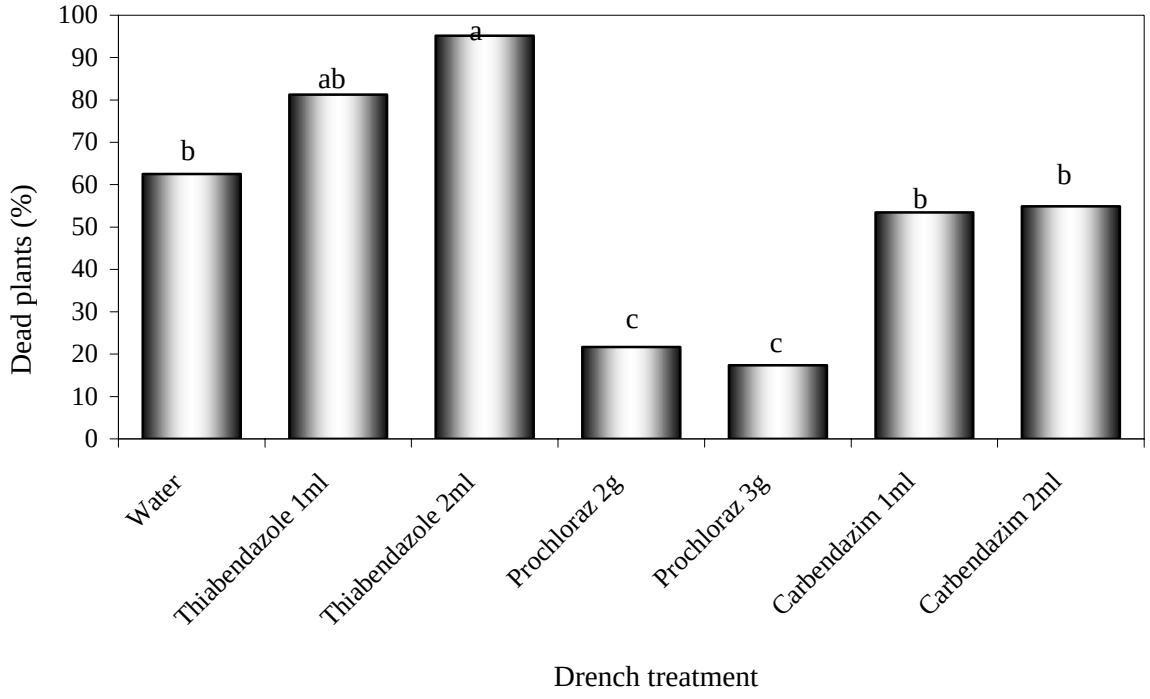
NB. Treatments with the same letter are not significantly different from one another (P=0.05).

Figure 17. Experiment 2. Incidence of dead plants inoculated with *F. avenaceum* at concentrations of either 10^2, 10^4, or 10^6 macrospores/ml and drenched with either Trichoderma, thiabendazole, carbendazim, prochloraz or water 2 or 7 days later.

Experiment 3

Significantly less seedlings died when drenched in the low or high rate of prochloraz with 21 and 17% of seedlings missing respectively compared to 62% of plants dead in the water treated. Carbendazim treatments reduced the incidence of missing plants, however was not significantly different to the untreated (Figure 18).

All treatments except thiabendazole reduced the number of missing seedlings, where 81 and 95% of dead seedlings were found in the thiabendazole treatments compared to the untreated. The cause of seedling death was due to phytotoxicity as seen in previous experiments, as no *F. avenaceum* was isolated from declining seedlings and minimal foot rot symptoms were observed in this chemical treatment compared to the others where *F. avenaceum* was constantly isolated from the crown and roots of seedlings.



NB. Treatments with the same letter are not significantly different from one another (P=0.05).

Figure 18. Experiment 3. Incidence of dead plants inoculated with *F. avenaceum* and drenched with fungicides.

Leek seedling drenches are effective in reducing seedling loss from *Fusarium* infection. The best chemicals applied as seedling drenches were prochloraz, fludioxonil, tebuconazole and carbendazim. The effect of these chemical treatments support the results seen in the *in vitro* studies where the best treatment was prochloraz and disc experiments where the most inhibitory treatments were prochloraz, carbendazim and thiabendazole + thiram. Thiabendazole was used as an alternative to thiabendazole + thiram, both having thiabendazole as the active ingredient. While no *Fusarium* developed, it had a phytotoxic effect on the seedlings. These chemicals will be field tested to determine their effect on natural and artificial infection of *Fusarium*.

Field trials

Field trials were undertaken in South Australia and Victoria to determine the incidence of *Fusarium* in leek plantings and to evaluate the efficacy of fungicide and biological treatments previously evaluated in the laboratory and greenhouse for *Fusarium* control. The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Objective: *To determine the incidence of Fusarium in leeks plantings, Lenswood 2001.*

Materials and methods

Two trials were undertaken at Lenswood to survey the incidence of *Fusarium* in plantings and investigate the level of *Fusarium* infection from seedlings. Both trials were planted in virgin ground.

Field trial 1

Leek seedlings cv. Missile were planted on February 26, 2001 in raised beds 1.65m wide. Three beds were planted consisting of four rows of seedlings 33cm apart with 13cm spacings between plants. Plots were replicated 6 times and 3 replicates of 80 plants were harvested and washed at 2 sampling times and assessed for incidence and severity of foot rot as described in the general materials and methods.

Field trial 2

Leek seedlings cv. Harpoon were planted on February 28, 2001 in raised beds 1.65m wide. Four beds of seedlings were planted as mentioned in trial 1. Plots were replicated 16 times and 4 replicates of 80 plants were harvested and washed at 3 sampling times and assessed for incidence and severity of foot rot as previously mentioned.

Results and discussion

Field Trial 1

Fusarium was isolated from crown and basal sections of infected plants at both assessments. Foot rot was found on 4.8% of leeks at the first assessment, increasing to 6.5% at harvest (Table 7). Severity ratings were below 1 and leeks were considered marketable.

Table 7. Field Trial 1. Incidence and severity of Fusarium foot rot, Block I, Lenswood 2001.

Assessment date	Incidence	Severity
September 17, 2001	4.79	0.33
October 16, 2001	6.46	0.46
LSD (P=0.05)	ns	ns

Where: ns= no significant difference.

Field Trial 2

Foot rot was observed on 10.9% of leeks in this planting at the first assessment and increased to 16.6% at harvest. The severity of foot rot increased at each assessment with 1.6% of the leek area infected with rot at the first assessment compared to 2.3% of the leek area infected at harvest (Table 8).

These trials indicated that leeks can become infected with *Fusarium* even though planted in virgin ground. Therefore surveys need to be carried out to evaluate whether *Fusarium* is entering plantings on the seedlings. The level of foot rot was higher on the variety Harpoon with 11% of leeks infected at the first assessment compared to 5% of Missile leeks. This supports the previously mentioned variety susceptibility trial where the variety Harpoon was found to be the most susceptible to foot rot infection compared to the other varieties tested.

Table 8. Field Trial 2. Incidence and severity of Fusarium foot rot, Block C, Lenswood 2001.

Assessment date	Incidence	Severity
September 17, 2001	10.94 b	1.59
October 16, 2001	11.88 b	1.99
November 12, 2001	16.56 a	2.34
LSD (P=0.05)	0.04	ns

Where: ns= no significant difference.
NB. Assessment dates with the same letter are not significantly different from one another (P=0.05).

Objective: *To determine the levels of Fusarium infection in leek plantings on commercial properties, Nairne, Langhorne Creek and Murray Bridge 2001.*

Field trials were undertaken to determine whether planting leek seedlings infected with *Fusarium* increased seedling mortality in the field and disease incidence at harvest. Leek seedlings cv. Harpoon, Missile, Admiral/Missile and Admiral obtained from 2 different nurseries were planted on 3 commercial properties at Nairne, Langhorne Creek and Murray Bridge.

Materials and methods

Leek seedlings at each location were planted into the field in a single raised bed 1.65m wide, which was separated into 8 plots of 282 plants. On 3 occasions throughout the growing season leeks were assessed for dead and missing plants. Plots were harvested between 103-162 days after planting, washed and assessed for the incidence and severity of foot rot as previously mentioned. Sections of diseased root and crown tissue were plated onto TWA and incubated at 25^{o}C for 7-10 days to determine the fungus associated with disease symptoms. The *Fusarium* isolated was not identified to species.

Prior to planting, 50 seedlings were sampled at random from each seedling batch and assessed for foot rot symptoms. From this sample, 5 plants were surface sterilised and root and crown tissue plated onto TWA and incubated for 7-10 days at 25^{o}C to determine whether fungi was present. The remaining 45 plants were planted into UC soil in 10cm pots and maintained in a greenhouse at 25^{o}C. Seedling mortality was recorded over a 12-14 week period.

Results and discussion

Fusarium was found in all leek seedlings collected at planting sites except for Admiral seedlings from nursery A. The highest incidence of *Fusarium* was found in Harpoon seedlings from nursery B with 18% of seedlings infected, with the lowest incidence being 3% in the Admiral/Missile seedlings from nursery A. The incidence of *Fusarium* in seedlings at planting corresponded to the *Fusarium* infection on leeks grown in the greenhouse and field, and where higher numbers of dead plants occurred there was also a higher number of plants with foot rot symptoms.

Variety

The incidence of dead plants was significantly higher in the variety Missile with 27% of leeks missing. This incidence level corresponded to the level of *Fusarium* infection at harvest with 34.7% of Missile leeks infected with foot rot. This level of death and infection was higher than expected, as only 4% of the seedlings were infected at planting. While the leek variety Harpoon also had a high leek mortality with 15-21% of leeks missing at harvest. The crop with 21% mortality had a higher incidence of infected seedlings at 18% compared to the Missile. This may indicate that Harpoon is a more susceptible variety than Missile. The lowest number of dead plants recorded was found in the Admiral/Missile leeks with 0.9% missing while the variety Admiral had the least foot rot with 5.5% of leeks infected (Table 10).

Nursery

Seedlings planted in the field from nursery A had significantly less dead plants at harvest than those plots planted with nursery B seedlings with 1.4 and 21.4% of seedlings missing from nursery A and B respectively. The level of mortality corresponded to foot rot infection with 11.4% and 33.1% of leeks infected with foot rot from nursery A and B respectively (Table 10).

Site

Differences were detected between trial site location. A higher incidence of dead plants and foot rot infection were found at Nairne with 24.1% of leeks missing and 41% of leek infected at harvest. The

lowest number of dead plants were found at Langhorne Creek with 5.9% of leeks dead which corresponded to the levels of foot rot found on leeks in this trial site at harvest where 14.7% were infected. Nairne has a heavier clay loam soil compared to the sandy soil at Langhorne Creek, which may influence the development of *Fusarium*.

Fusarium was isolated from plants with basal and crown rot symptoms suggesting that it is the main cause of disease and results indicate that the health of 10-12 week old seedlings is important in producing healthy plants at harvest.

Table 9. Incidence of Fusarium foot rot and dead plants from different varieties, nurseries and planting sites, 2001.

Variety	Nursery	Site	% infection at planting	% Dead plants after 14 weeks	Incidence at harvest (%)			
					Dead		Foot Rot	
Harpoon	B	2	n.a.	n.a.	24	ab	34.1	b
Harpoon	B	2	n.a.	n.a.	15	c	27	bc
Harpoon	B	1	n.a.	n.a.	20	bc	25.2	cd
Harpoon	B	3	18	15	21	b	46.8	a
Missile	B	3	4	10	27	a	34.7	b
Admiral/Missile	A	1	10	0	1	d	18.1	d
Admiral/Missile	A	1	3	3	0.4	d	10.2	e
Admiral	A	1	0	0	2	d	5.5	e
LSD (P=0.05)					sig		sig	

NB. Treatments with the same letter are not significantly different from one another (P=0.05).
Site: 1=Langhorne Creek, 2=Murray Bridge, 3=Nairne
Where: n.a. = Not available, sig. = Significant difference between incidence levels.

Table 10. Incidence of Fusarium foot rot and dead plants at harvest from different leek varieties, nurseries and planting sites, 2001.

		Incidence at harvest (%)	
		Missing/Dead	Fusarium Foot rot
Variety	Harpoon	20 b	32.7 a
	Missile	27 a	34.7 a
	Admiral	2.5 c	5.5 b
	Admiral/Missile	0.9 c	14.2 b
LSD(P=0.05)		sig	sig
Nursery	Nursery A	1.4 b	11.4 b
	Nursery B	21.4 a	33.1 a
LSD(P=0.05)		sig	sig
Site	Nairne	24.1 a	41 a
	Murray Bridge	19.7 b	30.4 b
	Langhorne Creek	5.9 c	14.7 c
LSD(P=0.05)		sig	sig

NB. Treatments with the same letter are not significantly different from one another (P=0.05).
Where: sig. = Significant difference between incidence levels.

Objective: *To evaluate fungicide or biological seedling drenches or fungigation treatments at planting for the control of Fusarium.*

Field trials were undertaken at the Lenswood Research Centre and on commercial properties at Murray Bridge, Nairne and Langhorne Creek to evaluate the efficacy of fungicide and biological control agents for the control of *Fusarium*. The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Murray Bridge 2001/02

Materials and methods

Leek seedlings cv. Missile were planted November 29, 2001 in raised beds 1.65m wide. Four rows of seedlings were planted per bed 33cm apart and seedlings spaced 13cm apart. The trial consisted of a single bed 125m long separated into treatment plots 5m long and replicated 4 times. Treatments included carbendazim (2ml/L), prochloraz (2.16g/L), Trichoderma (trichogrow) (10g/L) or Trichoderma (trichoflow) (10g/L) applied as a seedling drench or a fungigation treatment.

Plots were assessed for missing and dead plants 49 and 90 days after planting with 60 plants harvested from each plot 165 days after planting and the incidence and severity of foot rot assessed as previously described in the general material and methods.

Results and discussion

The number of dead leeks in this planting varied between 2.5% in carbendazim fungigation treatment plots to 5% in carbendazim drench plots. There were no significant differences between treatments and the number of dead plants (Figure 19).

Foot rot was found in all treatment plots with infection levels ranging from 2.5% in the carbendazim drench up to 4.4% in the carbendazim fungigation (Table 11). Severity was low with less than 1% of the leek area infected in all treatments. None of the treatments in this trial were effective in controlling Fusarium foot rot.

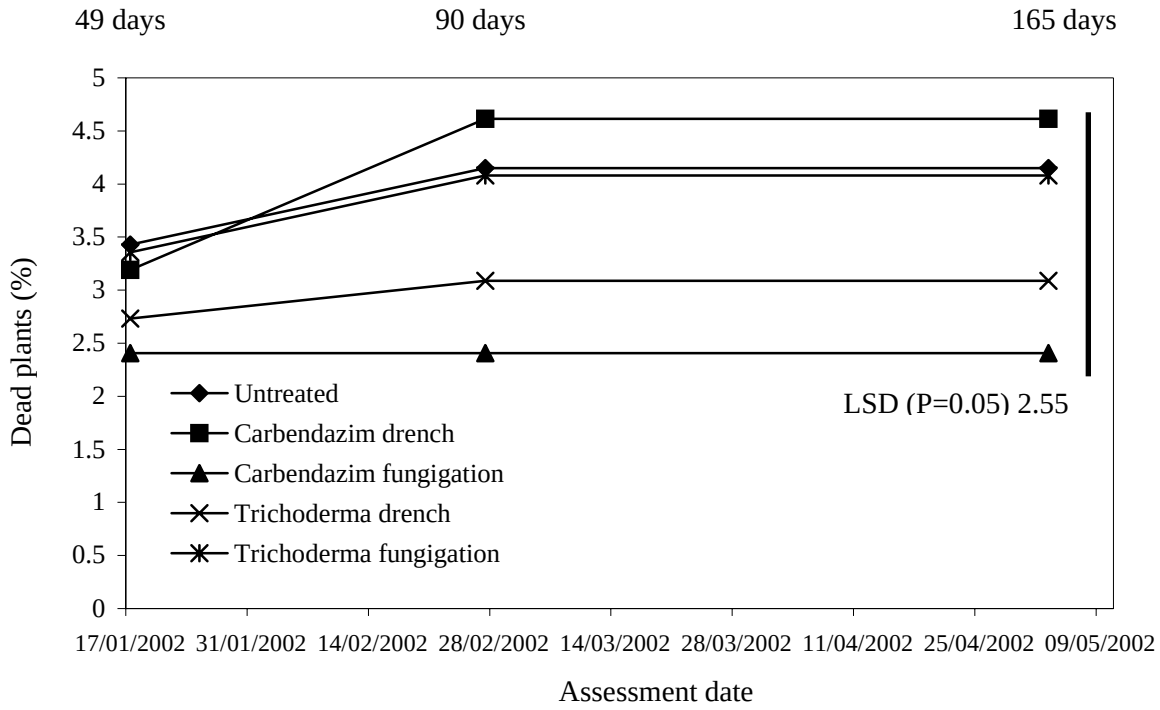


Figure 19. Effect of fungicide or biological treatments on the incidence of dead plants throughout the growing season, Murray Bridge 2001/02.

Table 11. Incidence and severity of Fusarium foot rot on leeks at harvest, Murray Bridge 2001/02.

Treatment	Fusarium Foot Rot	
	Incidence	Severity
Untreated	3.61	0.51
Carbendazim fungigation	4.42	0.67
Carbendazim drench	2.52	0.35
Trichoderma drench	3.37	0.45
Trichoderma fungigation	3.37	0.34
LSD (P=0.05)	ns	ns

Where: ns = there are no significant differences between treatments.

Langhorne Creek 2001/02

Materials and methods

Leek seedlings cv. Missile were planted on November 30, 2001 in a raised bed 1.65m wide. Three rows of seedlings were planted per bed 41cm apart with seedlings spaced at 13cm. The trial was undertaken on a single bed 125m long separated into 5m treatment plots and replicated 4 times at random along the bed. Fungicide treatments included carbendazim (2ml/L), prochloraz (2.16g/L), Trichoderma (trichogrow) (10g/L) or Trichoderma (trichoflow) (10g/L) applied as a seedling drench or fungigation treatment. Missing and dead plants were assessed throughout the growing season 18,

62 and 89 days after planting with 60 plants harvested from each plot 153 days after planting and assessed for foot rot as previously described in the general materials and methods.

Results and discussion

The incidence of dead plants increased throughout the season with significantly more dead plants found in the untreated plots (2.1%) missing compared to the treated plots (0.5-0.9%) 89 days after planting (Figure 20).

The incidence of foot rot was low with 3.1% of leeks infected in the untreated plots. No treatment significantly reduced the incidence of foot rot in this planting. The most effective treatment was carbendazim fungigation with 1.9% of leeks infected while a Trichoderma drench was not effective with 5% of leeks infected. The severity of basal rot remained low in all treatments with less than 1% of the leek area infected in all treatments except for the Trichoderma drench with 1.5% of the leek infected (Table 12).

While there was no statistical differences in levels of Fusarium foot rot between treatments, carbendazim numerically reduced infection and all treatments reduced the numbers of dead plants.

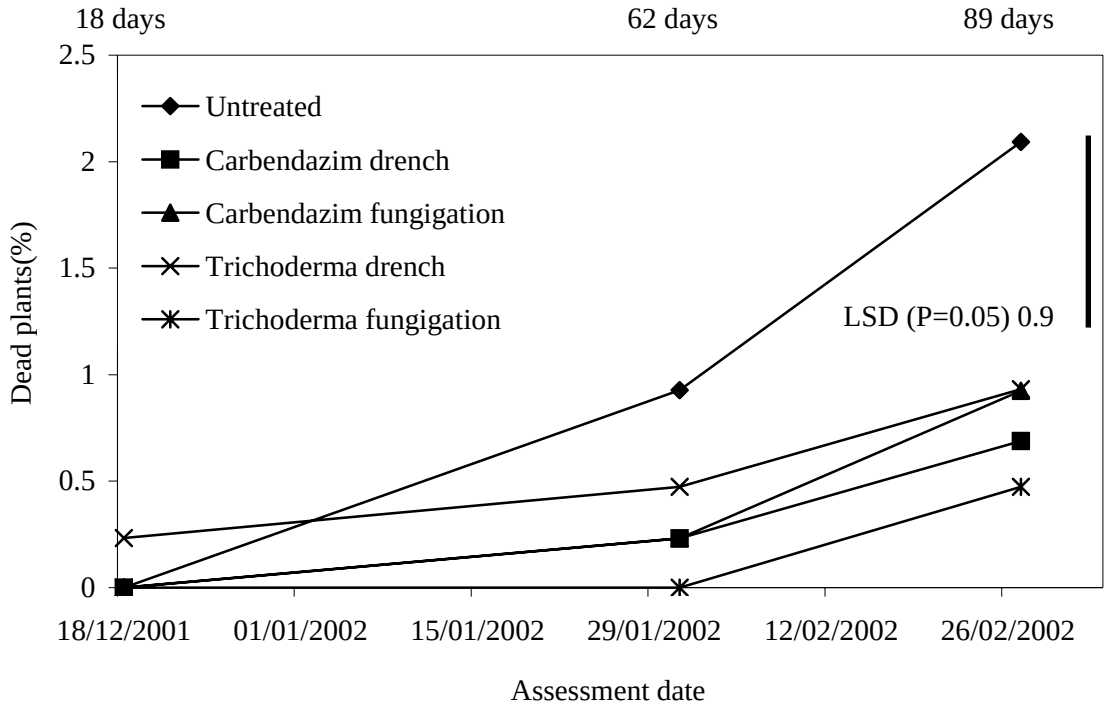


Figure 20. Effect of fungicide or biological treatments on the incidence of dead plants throughout the growing season, Langhorne Creek, 2001/02.

Table 12. Incidence and severity of Fusarium foot rot on leeks at harvest, Langhorne Creek 2001/02.

Treatment	Fusarium Foot Rot	
	Incidence	Severity
Untreated	3.13	0.48
Carbendazim drench	2.71	0.67
Carbendazim fungigation	1.9	0.48
Trichoderma drench	5	1.49
Trichoderma fungigation	4.19	0.94
LSD (P=0.05)	ns	ns

Where: ns = there are no significant differences between treatments.

Nairne 2002

Materials and methods

Leek seedlings cv. Missile were planted on February 4, 2002 in raised beds 1.65m wide. Four rows of seedlings were planted per bed 33cm apart and seedlings spaced at 13cm. The trial was arranged in a single bed 140m long separated into 5m treatment plots replicated 4 times at random along the bed.

Fungicide solutions of carbendazim (2ml/L), prochloraz (2.16g/L) Trichoderma (trichogrow) (10g/L) and Trichoderma (trichoflow) (10g/L) were applied to leek seedlings as drench or fungigation treatments. Plots were assessed for missing and dead plants at 30, 99 and 230 days and 60 plants harvested from each plot 231 days after planting and assessed for the incidence and severity of foot rot as previously mentioned in the general materials and methods.

Results and discussion

The number of dead plants increased throughout the growing season with 4.6% of plants dying in the untreated plots 230 days after planting. No fungigation or drench treatment significantly reduced the number of dead plants.

The incidence of foot rot was low with only 2.6% of plants infected in untreated plots at harvest (Table 13). Although the effects of fungicides were not statistically significant both carbendazim and Trichoderma were effective at reducing the number of dead plants and the level of Fusarium foot rot. Drenching seedlings was more effective the fungigation.

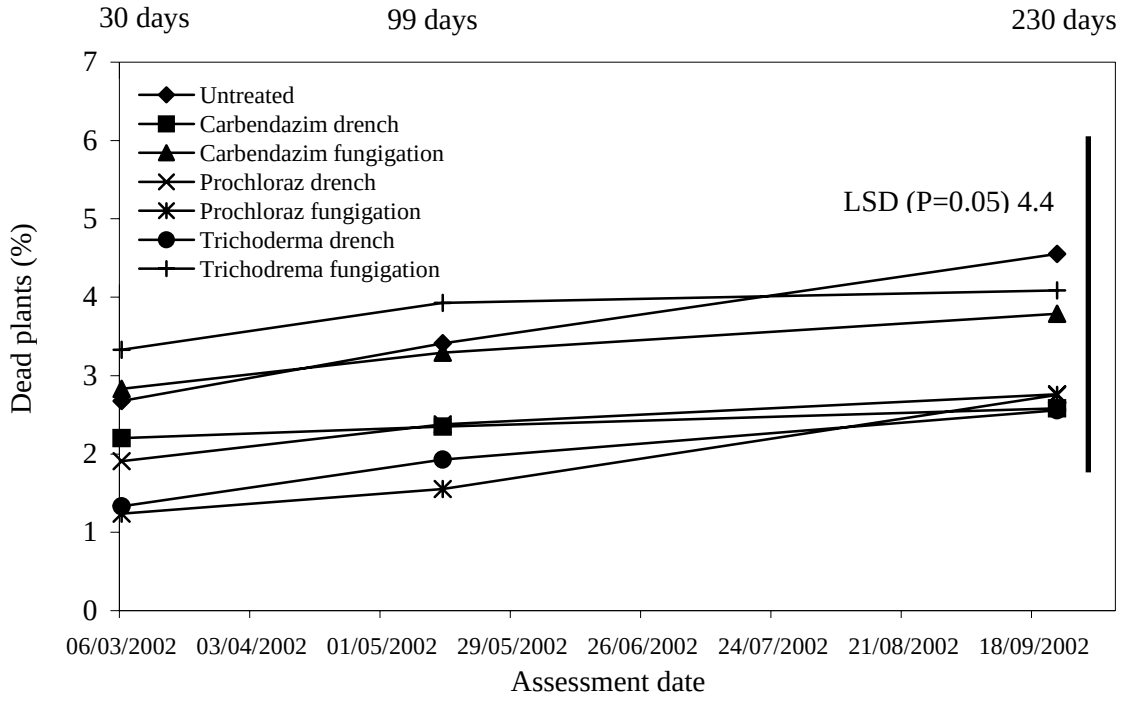


Figure 21. Effect of fungicide or biological treatments on the incidence of dead plants throughout the growing season, Nairne 2002.

Table 13. Incidence and severity of Fusarium foot rot on leeks at harvest, Nairne 2002.

Treatment	Fusarium Foot Rot	
	Incidence	Severity
Untreated	2.55 b	0.56
Carbendazim drench	1.95 b	0.38
Carbendazim fungigation	3.34 ab	0.42
Prochloraz drench	1.52 b	0.4
Prochloraz fungigation	5.09 a	1.43
Trichoderma drench	0.88 b	0.53
Trichoderma fungigation	2 b	0.13
LSD (P=0.05)	0.03	ns

NB. Treatments with the same letter are not significantly different from one another (P=0.05).
Where: ns = no significant differences were detected between treatments.

Lenswood 2002

Materials and methods

Two field trials were undertaken at the Lenswood Research Centre in 2002 planted in 2 separate blocks. Leeks in block 1 were planted into virgin soil while block 2 was planted with leeks the previous year that had been infected with Fusarium foot rot. Leek seedlings cv. Missile were planted on February 13, 2002 into beds 1.65m wide containing 4 rows of leeks planted 33cm apart and spaced at 13cm. Both trials were arranged in a randomised block design over 6 beds consisting of 7

treatments replicated 6 times. Seedlings were treated once at planting by either drenching speedling trays in 2 litres of fungicide or biological solution or water for 3 minutes or fungigating leeks after planting. Drench treatments applied were carbendazim (2ml/L), Trichoderma (trichoflow) (10g/L) and prochloraz (2.16g/L) and fungigation treatments applied were carbendazim (2ml/L), Trichoderma (trichogrow) (10g/L) and prochloraz (2.16g/L). Trials were assessed throughout the growing season for dead and missing plants and the incidence and severity of foot rot assessed at harvest as mentioned in the general materials and methods.

Results and discussion

Block 1

The incidence of dead plants increased throughout the growing season in all treatments. No significant differences were detected between treatments with the highest number of dead plants found in the carbendazim fungigation treatment where 5.1% of leeks were missing 251 days after planting. The lowest number of dead plants were found in treatments including fungigation with prochloraz and drenching with Trichoderma with 2.1 and 2.4% of plants missing respectively (Figure 22).

Foot rot was found in all treatments at harvest with 11.7% of leeks infected in the untreated plots. Infection levels were significantly reduced when seedlings were treated with either a carbendazim drench (5.6%), an prochloraz drench (2.3%) or prochloraz fungigation (5.6%). Similar trends were seen with severity levels with carbendazim and prochloraz reducing the severity of infection (Table 14).

Block 2

The incidence of dead plants over the season was the greatest in the Trichoderma and carbendazim fungigation treatments with 10 and 7% of leeks missing 250 days after planting compared to the untreated plots with 6.1%. Both prochloraz treatments had significantly less dead plants than the untreated with 2.4% of leeks missing in both prochloraz treatments (Figure 23).

The incidence of Fusarium foot rot was high in this planting with 30% of leeks infected in untreated plots. Carbendazim and prochloraz drench and fungigation treatments significantly reduced the level of foot rot at harvest. Carbendazim applied as a drench or fungigation reduced infection to 13.2 and 15.8% respectively while prochloraz reduced infection levels to 6 and 5% in the drench and fungigation treatments (Table 15). The Trichoderma treatments had no effect in reducing foot rot infection. Similar results were seen with foot rot severity levels at harvest. The prochloraz treatments reduced the area of infection to 1% compared to 11% in the untreated and Trichoderma treatments. Carbendazim treatments were also effective with 5% of the leek area infected at harvest.

Overall both carbendazim and prochloraz reduced the levels of Fusarium foot rot and numbers of dead plants with prochloraz providing better control than carbendazim. Disease levels were lower in the virgin ground and drenches were more effective. However in old ground the fungigation treatment were more effective, indicating that it had an effect on the soil borne inoculum levels as well as reducing seedling infection pre planting.

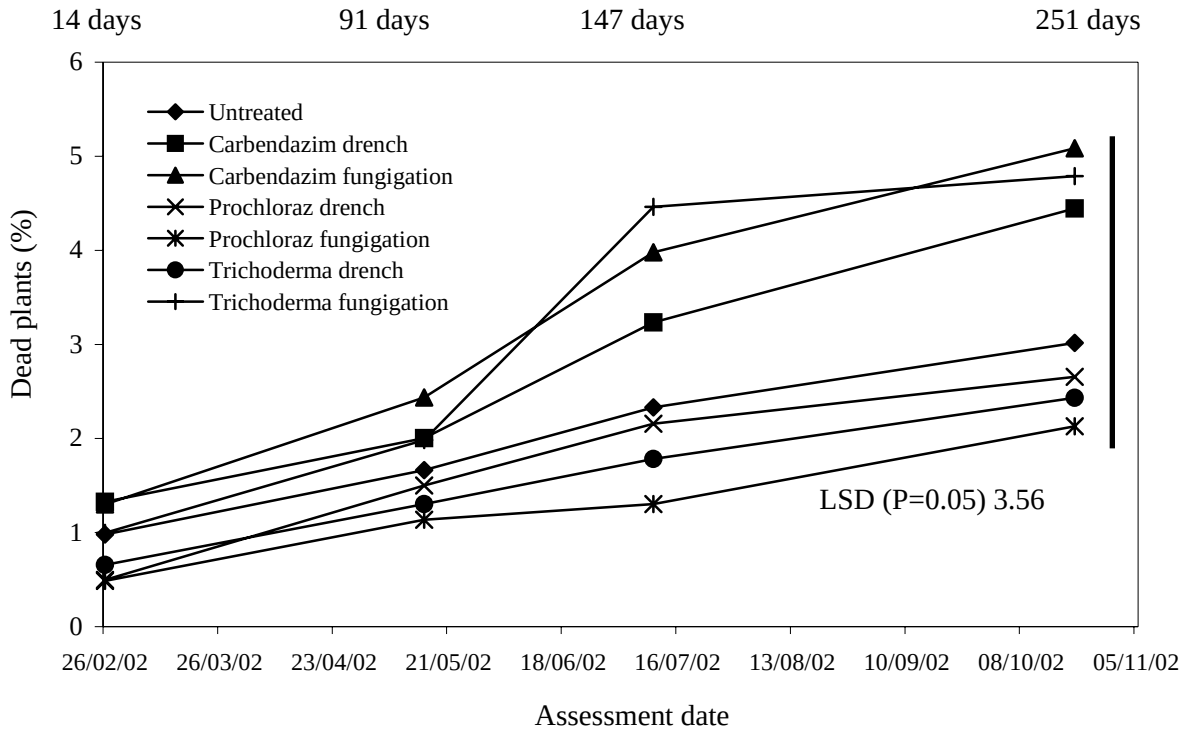


Figure 22. Effect of fungicide or biological treatments on the incidence of dead plants throughout the growing season, Lenswood (Block 1), 2002.

Table 14. Incidence and severity of Fusarium foot rot, Block 1, Lenswood 2002.

	Fusarium Foot Rot	
	Incidence	Severity
Untreated	11.73 a	3.42 a
Carbendazim drench	5.61 bc	1.29 bc
Carbendazim fungigation	10.99 a	2.87 a
Prochloraz drench	2.28 c	0.37 c
Prochloraz fungigation	5.58 bc	1.12 bc
Trichoderma drench	9.68 ab	2.33 ab
Trichoderma fungigation	11.55 a	3.09 a
LSD (P=0.05)	0.004	0.001

NB. Treatments with the same letter are not significantly different from one another (P=0.05).

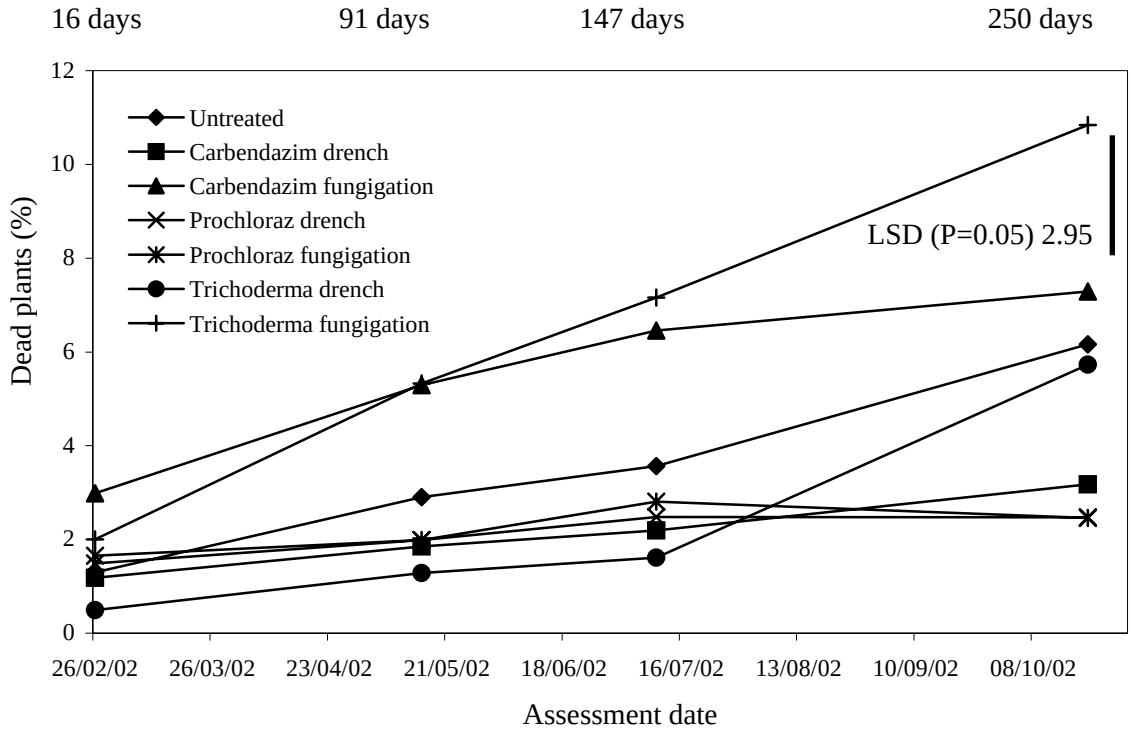


Figure 23. Effect of fungicide or biological treatments on the incidence of dead plants throughout the growing season, Lenswood (Block 2), 2002.

Table 15. Incidence and severity of Fusarium foot rot, Block 2, Lenswood 2002.

Treatment	**Fusarium foot rot**	
	Incidence	Severity
Untreated	30.06 a	10.96 a
Carbendazim drench	13.23 b	5.06 bc
Carbendazim fungigation	15.79 b	4.62 bc
Prochloraz drench	5.96 b	1.26 c
Prochloraz fungigation	4.87 b	1.15 c
Trichoderma drench	35.65 a	11.45 a
Trichoderma fungigation	30.19 a	9.19 ab
LSD (P=0.05)	0.0001	0.0003

NB. Treatments with the same letter are not significantly different from one another (P=0.05).

Lenswood 2004

Objective: *To evaluate the efficacy of fungicides applied to artificially inoculated leeks seedlings at planting for the control of Fusarium, Lenswood 2004.*

Materials and methods

Leek seedlings cv. Tokyo obtained from a commercial nursery were artificially inoculated with various pathogenic *Fusarium* isolates (Table 2) 7 days prior to planting by spraying a suspension containing 8.2×10^5 macrospores/ml over the seedlings. Seedlings were maintained in a shade house for 7 days before planting on February 5, 2004. Seedling trays were drenched in solutions of either thiabendazole (2ml/L), prochloraz (2.16g/L), carbendazim (2ml/L) or water just prior to planting. Once planted seedlings were irrigated with water or one of the 3 fungicides previously mentioned (Table 16). Leeks were assessed monthly and harvested 6 months after planting where they were washed and assessed for incidence and severity of foot rot as previously mentioned in the general materials and methods.

Table 16. Drench and fungigation treatments applied to leek seedlings, Lenswood 2004. The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Drench	Fungigation
Water	-
-	Water
Water	Water
Water	Carbendazim
Water	Prochloraz
Water	Thiabendazole
Carbendazim	Water
Carbendazim	Carbendazim
Carbendazim	Prochloraz
Carbendazim	Thiabendazole
Prochloraz	Water
Prochloraz	Carbendazim
Prochloraz	Prochloraz
Prochloraz	Thiabendazole
Thiabendazole	Water
Thiabendazole	Carbendazim
Thiabendazole	Prochloraz
Thiabendazole	Thiabendazole

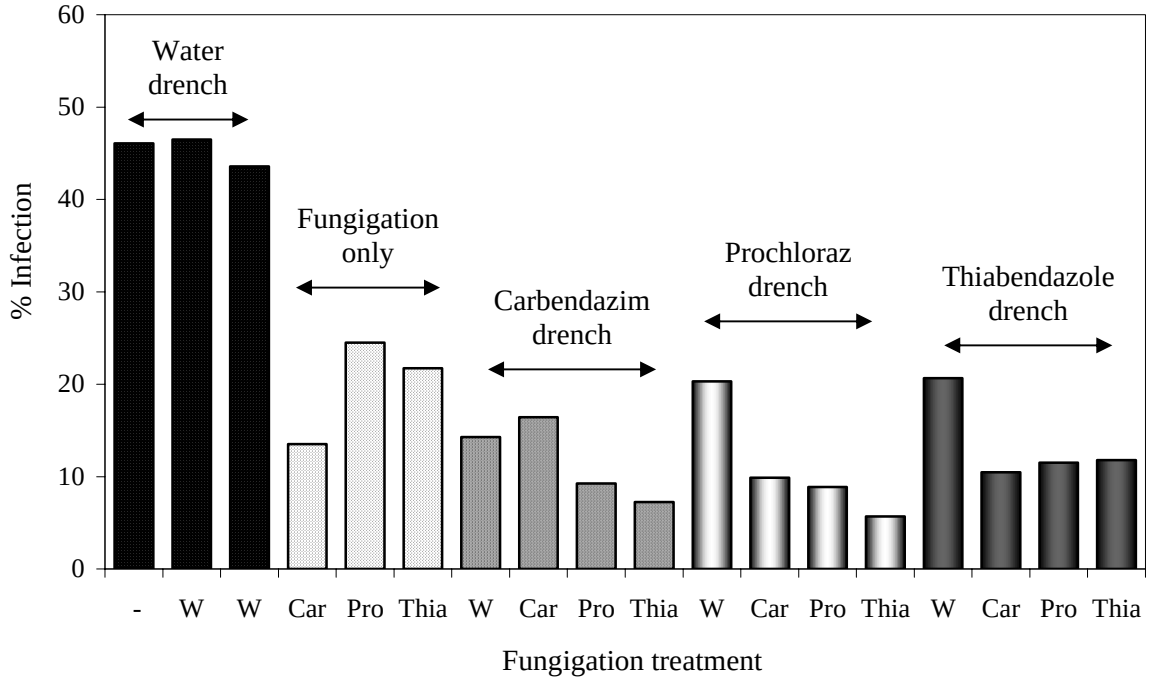
Results and discussion

In this trial Fusarium foot rot developed in 43 and 46% of plots drenched or fungigated with water alone. Significantly less disease developed in the plots treated with fungicide. These results show that drench or fungigation treatments alone were not as effective as a combined treatment. However, carbendazim applied as a fungigation treatment alone was the most effective single treatment.

The best treatment for the control of foot rot was an prochloraz drench followed by a fungigation treatment of thiabendazole as 6% of leeks were infected compared to 43% of leeks in untreated plots at harvest (Figure 24). A carbendazim drench followed by a thiabendazole fungigation treatment also provided good control with 7% of leeks infected with foot rot.

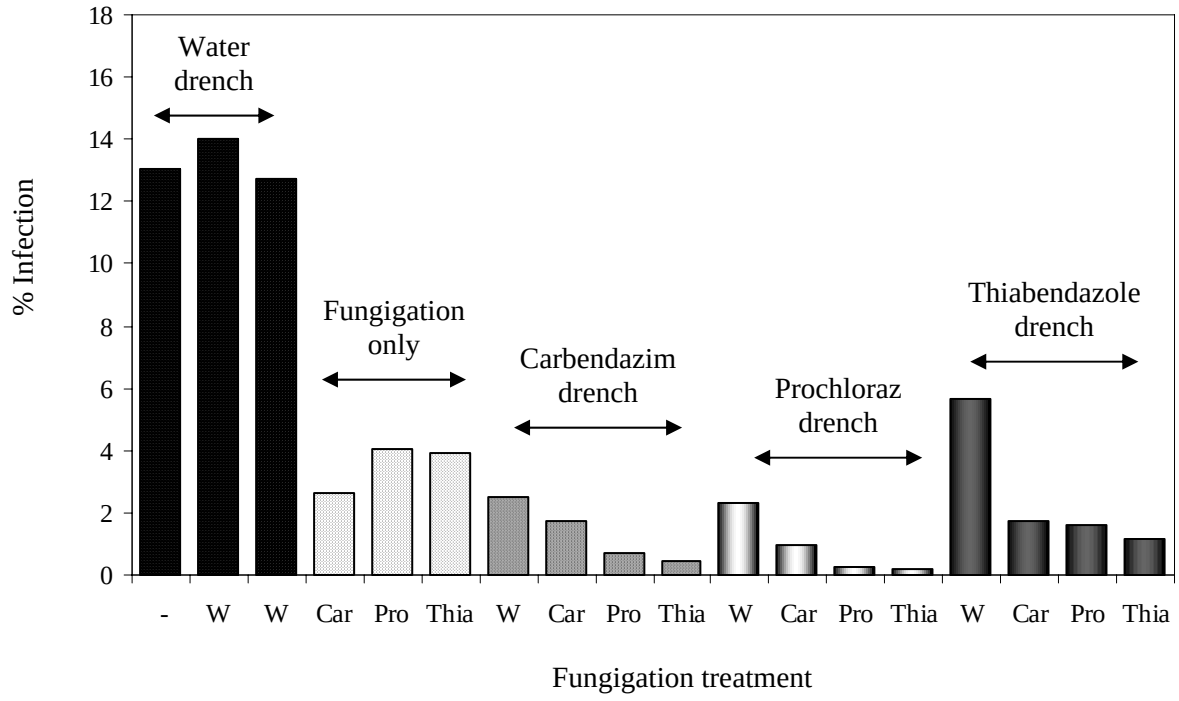
The severity of basal infection was significantly higher in the untreated plots compared to those treated at planting with 12-14% of the leek infected with foot rot at harvest. The lowest severity of foot rot infection was found on leeks treated with an prochloraz drench and thiabendazole fungigation with 0.2% of the leek area infected (Figure 25).

Overall the best control of Fusarium foot rot was achieved by drenching seedlings in prochloraz followed by fungigation with thiabendazole or prochloraz.



Where: W = Water, Car = Carbendazim, Pro = Prochloraz and Thia = Thiabendazole.

Figure 24. Incidence of Fusarium foot rot on artificially inoculated leeks, Lenswood 2004.



Where: W = Water, Car = Carbendazim, Pro = Prochloraz and Thia = Thiabendazole.

Figure 25. Severity of Fusarium foot rot on artificially inoculated leeks, Lenswood 2004.

Objective: *To evaluate the efficacy of fungicides applied as a drench prior to planting for the control of Fusarium, Victoria 2004.*

Materials and methods

Two field trials were set up on growers properties in Victoria at Clyde and Cranbourne. Leek seedlings cv. Nova were drenched for 3 minutes in 10 litre solutions of either carbendazim, prochloraz, tebuconazole or thiabendazole (Table 17) in August 2004. Only the high rates of fungicide were applied to seedlings at Cranbourne as the trial plot was smaller. The trials were assessed throughout the growing season for dead plants and were harvested in December and assessed for the incidence and severity of foot rot as mentioned previously.

Table 17. Fungicides applied as seedling drenches before planting, Victoria 2004. The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Fungicide	Rate	Clyde	Cranbourne
Carbendazim	1ml/L	✓	-
Carbendazim	2ml/L	✓	✓
Prochloraz	2g/L	✓	-
Prochloraz	3g/L	✓	✓
Tebuconazole	1ml/L	✓	-
Tebuconazole	2ml/L	✓	✓
Thiabendazole	2ml/L	✓	✓
Untreated	-	✓	✓

Results and discussion

No differences were detected between treatments and the number of dead plants at harvest. Although no significant difference was detected, the number of dead plants in the untreated was 14% compared to the carbendazim drench where no dead plants were found (Table 18).

Low levels of *Fusarium* were detected in the field at Cranbourne with 1.3-2.4% of leeks infected with foot rot (Table 19). While the differences were not statistically significant, leeks treated with carbendazim had the lowest incidence and severity of foot rot.

No disease was detected in the trial carried out at Clyde.

Table 18. Incidence of dead and missing leeks at harvest, Cranbourne Victoria, December 2004.

Drench	Rate	Dead/missing plants at harvest (%)
Water	-	14.1
Carbendazim	2ml/L	0
Prochloraz	3g/L	4.6
Tebuconazole	2ml/L	6.4
Thiabendazole	2ml/L	4.4
LSD (P=0.05)		ns

Where: ns= no significant difference.

Table 19. Incidence and severity of Fusarium foot rot on leeks, Cranbourne Victoria, December 2004.

Drench	Rate	Fusarium Foot Rot	
		Incidence	Severity
Water	-	2.09	0.05
Carbendazim	2ml/L	1.31	0.03
Prochloraz	3g/L	1.6	0.04
Tebuconazole	2ml/L	1.46	0.06
Thiabendazole	2ml/L	2.43	0.09
LSD (P=0.05)		ns.	ns.

Where: ns = no significant differences.

Conclusions

Fusarium foot rot caused mainly by *Fusarium avenaceum* and *F. oxysporum* can cause significant damage to leek plantings in Australia. Foot rot was observed as either a crown rot found around the base of leek stems or a rot affecting the basal plate. The two types of rot were commonly found together and both contributed to reducing the marketability of leeks.

The optimum temperature for mycelial growth of *Fusarium* is 20-25^{o}C. Leek varieties Missile, Harpoon, Admiral, Amundo and Nova were susceptible to infection. Leeks of all ages can become infected, with infection levels increasing with damage to seedlings, and high or low concentrations of inoculum can cause infection.

Planting disease free seedlings is an important factor for the control of *Fusarium* as an increase in seedling infection at planting increases disease incidence at harvest. This however is difficult to evaluate as seedlings can be infected without showing symptoms.

In vitro experiments showed that the fungicides prochloraz, fludioxonil, carbendazim, tebuconazole and thiabendazole + thiram controlled *Fusarium*. The results were confirmed in leek disc and greenhouse experiments. Although no control method completely eliminated *Fusarium* from the field, applying either an prochloraz drench or fungigating with carbendazim at planting reduced the incidence of *Fusarium* at harvest.

Bacterial blight

Introduction

Bacterial blight is a major disease of leeks in all leek growing areas of Australia. The bacteria appears as brown leaf lesions surrounded by a narrow yellow halo and longitudinal water soaked tissue extending as a narrow strip from the leaf tip to the crown and brown longitudinal stripes on leek stems. Infected leaves are often curled and light green in colour. This disease not only causes damage to seedlings resulting in seedling loss and poor establishment but severe infection causes stem staining and rot on mature plants. Experiments were carried out to identify management strategies for the control of this disease, to determine if the disease is seed borne and if so to evaluate control methods.

General materials and methods

Selective media: Three selective media were used for *Pseudomonas*. Nutrient Agar (NA) was used to maintain colonies and grow inoculum. King's B (KB) media was used to identify fluorescing colonies and Sucrose Nutrient Agar (SNA) to identify levan colonies characteristic of *Pseudomonas*.

Detection and isolation of bacteria: Diseased tissue with leaf lesions and leaf streaking were tested for bacterial streaming. A 5mm section of plant material was taken from the margin of infected tissue, placed in a drop of water on a microscope slide and examined microscopically for the presence of bacteria. When present, the bacteria suspension was streaked onto NA and KB and incubated at 28^{o}C for 48 hours. Plates were then examined under UV (ultra violet) light to determine the presence of fluorescent *Pseudomonas*.

Inoculum Preparation: Inoculum for greenhouse experiments was produced by removing a loopful of a known *Pseudomonas* isolate (DAR 75555) and placing into 9ml of nutrient broth. This solution was then placed on an automatic stirrer for 48 hours at 25^{o}C. After this time the suspension was removed and amended using a spectrometer to an absorbance of 1 (490 λ). Bulk inoculum for field trials was prepared in the same way however 3 loopfuls of bacteria were placed into 100ml of nutrient broth.

Field trial location: Field trials for seed borne experiments were carried out in 2003 at the Lenswood Research Centre located 30km east of Adelaide, South Australia.

Leek disc assessment: Incidence and severity of bacterial rot on discs was assessed using a 0-4 rating scale where 0 = no disease, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100% of the leek discs infected.

Seed germination: Leek seed was surface sterilised in 70% ethanol before being dried in a laminar flow cabinet. Seeds were germinated in 10cm pots containing a sterile 50:50 perlite: vermiculite mix, watered and maintained in a growth room at 25^{o}C. After 2 weeks seedlings were transferred into larger pots or speedling trays for experiments.

Chemical control: Various fungicides and sanitisers were evaluated for their efficacy for the control of internal seed borne *Pseudomonas* (Table 20).

Table 20. Chemicals evaluated for the control of Bacterial blight. The chemicals used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Chemical	**Manufacturer**	**Active Ingredient**
Copper oxychloride®	Shell Australia	500g/Kg copper oxychloride
Kocide®	Griffin Corporation Aust Pty Ltd	500g/Kg copper hydroxide
Dithane®	Dow Agrosciences Aust	750g/Kg mancozeb
Ridomil Gold MZ®	Syngenta	640g/Kg mancozeb + 40g/Kg metalaxyl
Ridomil Gold 480 EC®	Syngenta	480g/Kg metalaxyl-M
Hydrogen Peroxide®	Sigma Pharmaceuticals Ltd	3% peroxide of hydrogen
Sodium carbonate®	Ajax Chemicals	Na_2CO_3 (MW=105.99)
Potassium permanganate	Ace Chemical company	$KMnO_4$ (MW=158.04)
Chlordox active®	Australian Agribusiness marketing	200g/L chlorine dioxide
Swimming pool chlorine®	Asia Pacific Specialty Chemicals Ltd	125g/L hypochlorite
Vitaclean®	Castrol Australia Pty Ltd	20g/L sodium hydroxide
Proxitane®	Solvay Interox Pty Ltd	50g/Kg peroxyacetic acid, 250g/L hydrogen peroxide, 75g/Kg acetic acid

Laboratory experiments

Leek disc experiments

Experiments were undertaken to screen chemicals for the potential to control *Pseudomonas* using the leek disc technique. Experiments were also set up to determine varietal susceptibility.

Objective: *To determine the efficacy of various chemicals for the control of Pseudomonas syringae pv. porri.*

Materials and methods

Two isolates of *Pseudomonas syringae* pv. *porri* collected from the Adelaide Hills were used for this experiment. Leek discs approximately 1cm thick were surface sterilised as previously described. Five discs for each treatment were placed in 200ml of a chemical solution for 3 minutes before being placed on trays containing a moistened chux and paper towel. Treatments included either copper oxychloride (2g/L), mancozeb (1.25g/L), mancozeb + metalaxyl (4g/L) or metalaxyl-M (2.08ml/L). After drying 100µl of a bacteria suspension was pipetted onto each disc and trays sealed in plastic bags. Trays were incubated for 11 days at room temperature and the incidence and severity of bacterial infection assessed as mentioned previously.

Results and discussion

The incidence of infection was similar for both isolates tested. Copper oxychloride, mancozeb + metalaxyl and metalaxyl-M provided some level of control of *Pseudomonas*, with 40-60% of discs infected with bacteria. The severity of infection on discs treated with isolate A (0.8 to 1.8%) was not significantly different to the untreated (2.2%). For isolate B the severity of infection on discs was significantly different in the copper oxychloride, metalaxyl-M and mancozeb + metalaxyl treatments with 0.6% of the disc infected compared to 2.6% in the untreated. Mancozeb had little inhibitory effect on either isolate.

Table 21. Incidence and severity of *Pseudomonas* on leek discs.

	Isolate A		Isolate B	
Treatment	**Incidence**	**Severity**	**Incidence**	**Severity**
Negative control	0 b	0 b	0 b	0 c
Positive control	100 a	2.2 a	100 a	2.6 a
Copper oxychloride	60 ab	0.8 ab	40 ab	0.6 bc
Mancozeb	100 a	1.8 ab	100 a	1.6 ab
Metalaxyl-M	80 a	1.4 ab	40 ab	0.6 bc
Mancozeb + metalaxyl	60 ab	1.2 ab	60 ab	0.6 bc
LSD (P=0.05)	75.3	1.8	79.8	1.3

N.B. Treatments with the same letter are not significantly different from one another (P=0.05).

Objective: *To determine the susceptibility of different leek varieties to Pseudomonas syringae pv. porri.*

Materials and methods

Leek seed of the varieties Missile, Harpoon, Nova and Admiral were germinated as previously described. After 2 weeks seedlings were transferred into 10cm pots containing UC soil and maintained in the greenhouse for a further 12 weeks at 25^{o}C. Inoculum was prepared as previously described and five plants per variety were inoculated with 500µl of bacteria suspension using a hypodermic syringe to inject the plant stems approximately 8cm above soil level. Immediately after inoculation, plants were placed in a mist tent at 100% humidity for 7 days, then removed and maintained in a greenhouse at 25^{o}C and assessed 14 and 49 days after inoculation for leaf symptoms and dead plants.

Results and discussion

Symptoms typical of *Pseudomonas syringae* pv. *porri* infection developed in all inoculated plants after 14 days. Brown leaf spotting, leaf curling and yellow streaking down the centre or edge of leaves developed in all four varieties. Infection was systemic and moved from the point of infection to leaf tissue and 10-15cm down the stem causing plants to wither and die.

All leek varieties tested were susceptible to bacterial infection with symptoms developing by 14 days after infection. No plants died after 14 days but 49 days after inoculation 50% of leeks cv. Admiral were dead. The least susceptible variety of leek was Nova with only 10% of leeks dead with 20% of leeks dead in Missile and Harpoon cultivars 49 days after inoculation.

Table 22. Incidence of bacterial symptoms and dead leeks 14 and 49 days after inoculation.

Variety	Leaf Curl	Yellow Streaking	Leaf Lesions	Dead	
	14 days	14 days	14 days	14 days	49 days
Missile	80	90	50	0	20
Harpoon	80	40	50	0	20
Nova	70	70	60	0	10
Admiral	70	60	30	0	50
LSD (P=0.05)	ns	ns	ns	ns	ns

Where: ns = no significant differences detected between treatments (P=0.05).

Field Trials

Research overseas reported that *Pseudomonas syringae* pv. *porri* is seed borne in leeks (6,7). Experiments were undertaken to determine if the bacteria could be isolated from seed used in Australia and whether *Pseudomonas* infected plants produce the infected seed.

Objective: *To demonstrate that Pseudomonas syringae pv. porri is seed borne.*

Materials and methods

The stalks of seed heads on 7 month old leek plants cv. Missile were inoculated with an isolate of *Pseudomonas syringae* pv. *porri*. A 1ml bacterial suspension was injected into the seed stalk of 72 plants using a hypodermic syringe. Sterile water was injected as a control treatment into a further 72 plants. Plastic bags sprayed inside with water to provide humidity were placed over the seed heads and sealed. These bags were removed 48 hours later. Plants were assessed after 10 days for stem lesions on the seed stalk and 8 weeks later the seed heads were removed, dried and the seed recovered and stored.

Thirty leek seeds collected from seed heads artificially inoculated with *Pseudomonas syringae* pv. *porri* were immersed in 70% ethanol for 3 minutes and macerated in 10ml of sterile water using a mortar and pestle. A 100µl suspension was then transferred onto the selective media KB and SNA for the isolation of *Pseudomonas*. Concentrations ranging from neat to 10^{-4} were spread over the plates and dried for 5 minutes in a laminar flow cabinet. After drying, plates were incubated at 28^{o}C for 48 hours before being assessed for the presence of fluorescing and levan colonies characteristic of *Pseudomonas.*

Results and discussion

Bacterial blight symptoms including brown lesions and water soaked tissue developed on 95 to 98% of seed stalks 10 days after inoculation (Pictures 2, 3 & 4) whereas no symptoms developed on the water inoculated leeks (Table 23).

Pseudomonas syringae pv. *porri* was detected in leek seeds collected from plants inoculated with the bacteria 8 weeks previously. These results show that the bacteria move systemically since the leek plants were injected with bacteria on the seed stalk, approximately 20-30cm below the seed head.

63% of seed lots tested positive for *Pseudomonas.* Prolific fluorescent colonies were observed on KB with the concentrations of fluorescent colonies on KB and levan colonies on SNA ranging from 10 – 1000 cfu's/ml in the 30 seeds.

Table 23. Incidence of lesions and water soaked tissue on leek seed stalks inoculated with *Pseudomonas.*

	Incidence (%)	
	Brown lesion	Water soaked tissue
Inoculated	95.2	98.8
Not inoculated	0	0

Picture 2, 3 & 4. Bacteria lesions on leek seed stalks inoculated with *Pseudomonas syringae* pv. *porri*, Lenswood 2003.

Seed Treatments

Previous experiments showed that *Pseudomonas* persisted in leek seed and that the bacteria was transferred from infected plants to seed. Studies were undertaken to determine if seed soaks reduce the level of *Pseudomonas* in seed and if the treatments inhibited seed germination.

Objective: *To determine the effect of hot water seed soaks on the control of Pseudomonas in leek seed and its effect on germination.*

Materials and methods

Batches of 30 seeds each previously collected from seed heads of artificially inoculated plants, were replicated 4 times and immersed in a water bath at either 50^{o}C for 25, 30 or 35 minutes or for 25 minutes at 52 or 55^{o}C. Another 250 leek seeds per treatment were soaked in hot water for germination testing. Seed was dried, surface sterilised in 70% ethanol for 3 minutes, dried and macerated in 10ml of sterile water using a mortar and pestle. Serial dilutions of seed solutions ranging from 10^{-2} to 10^{-4} were plated onto KB and SNA and incubated for 48 hours at 28^{o}C and assessed for fluorescent and levan colonies. Leek seed was germinated by placing 250 seeds onto germination paper and assessed after 2-3 weeks.

Results and discussion

Pseudomonas colonies developed in (a) 25% of samples treated for 25 minutes at 50^{o}C, (b) 12.5% of samples treated at 50^{o}C for 30 minutes and (c) 6% of samples treated at 52^{o}C for 25 minutes. Soaking seed in water at 50^{o}C for 35 minutes and 55^{o}C for 25 minutes completely eliminated *Pseudomonas* in seed.

Seed germination was severely inhibited by soaking in hot water at 50^{o}C with temperatures of 52^{o}C and over inhibiting germination completely (Table 24).

Table 24. Effect of hot water seed soaks for the control of *Pseudomonas*.

Temperature (^{o}C)	Timing (minutes)	Control achieved	Germination (%)
50	25	-	0.8
50	30	+	10
50	35	+++	3.6
52	25	+	0
55	25	+++	0
Untreated	-	-	22.4

Where: - no reduction on fluorescing colonies, + minimal reduction, ++ high level of colony reduction +++ complete elimination of colonies.

Objective: *Evaluation of various chemical seed soaks on the control of Pseudomonas seed infection.*

Materials and methods

Leek seed cv. Tiana RZ and Amundo artificially inoculated with *Pseudomonas* were used for these experiments. Seed was weighed into 13.5g lots and a bacterial suspension containing 10^8 cfu's/ml added to the seed before being agitated on an orbital shaker for 2 hours. Seed was then removed and dried to its original weight in a laminar flow cabinet.

Four experiments were undertaken evaluating various chemicals as outlined in Table 25, 26, 27 and 28. Batches of 100 seeds each were placed into nylon bags and soaked in the various chemicals for 5, 15 or 30 minutes, with 4 replicates of each treatment. The control was inoculated seed soaked in sterile water. Two replicates of the treated seed was surface sterilised in 70% ethanol for 3 minutes and then crushed in 10ml of sterile water using a mortar and pestle. The suspension was diluted to concentrations ranging between 10^{-3} and 10^{-6} and 100µl aliquots plated onto KB and SNA. After 48 hours incubation at 28^{o}C plates removed and the number of levan and fluorescent colonies counted. The other 2 replicates were germinated as mentioned in the general materials and methods.

Results and discussion

Experiment 1

No soak treatment completely eliminated bacteria from the seed. The number of colonies developing in dilutions from crushed seed were reduced when seed was treated with either 3% hydrogen peroxide for 15 minutes, 2% sodium carbonate for 5 minutes, 1% potassium permanganate or the 1% potassium permanganate and Tween 20 solution for 30 minutes. Colony numbers were not reduced when seed was soaked in 3% hydrogen peroxide for 5 minutes (Table 25).

Germination levels were not reduced by the chemical soaks when compared to seed soaked in water. In the 2% sodium carbonate and potassium permanganate treatments germination was 10% or more higher than that of the control (Table 25).

Table 25. Effect of seed soaks for the control of *Pseudomonas*.

Treatment	Timing	Control achieved	Germination (%)
Hydrogen peroxide 3%	5 minutes	-	51
Hydrogen peroxide 3%	15 minutes	++	52
Sodium carbonate 2%	5 minutes	++	65
Potassium permanganate 1%	30 minutes	++	59.3
Potassium permanganate 1% plus 1% Tween 20	30 minutes	++	52.3
Sterile water	5 minutes	-	52
Sterile water	15 minutes	-	54.3
Sterile water	30 minutes	-	54.6
LSD (P=0.05)			ns

Where: - no reduction on fluorescing colonies, + minimal reduction, ++ high level of colony reduction +++ complete elimination of colonies.
Where: ns = there are no significant differences between treatments.

Experiment 2

While no treatment completely inhibited bacteria, seed soaked in either 3% hydrogen peroxide for 15 minutes or 2% sodium carbonate for 5 minutes were the most effective in reducing the number of fluorescing bacteria colonies. Seed soaks of 3% hydrogen peroxide for 5 minutes and 2% sodium carbonate for 15 minutes reduced the number of fluorescing colonies however they were not as effective as those previously mentioned in experiment 1 (Table 26).

Germination was not inhibited with any treatment (Table 26).

Table 26. Effect of seed soaks for the control of *Pseudomonas*.

Treatment	Timing	Control achieved	Germination (%)
Hydrogen peroxide 3%	5 minutes	+	83.5
Hydrogen peroxide 3%	15 minutes	++	82
Sodium carbonate 2%	5 minutes	++	82
Sodium carbonate 2%	15 minutes	+	77
Potassium permanganate 1%	30 minutes	-	88.5
Potassium permanganate 1% plus 1% Tween 20	30 minutes	-	70.5
Sterile water	5 minutes	-	77
Sterile water	15 minutes	-	77.5
Sterile water	30 minutes	-	77
Un-inoculated Control Seed	-	-	87
Inoculated Control Seed	-	-	69.5
LSD (P=0.05)			ns

Where: - no reduction on fluorescing colonies, + minimal reduction, ++ high level of colony reduction +++ complete elimination of colonies.
Where: ns = there are no significant differences between treatments.

Experiment 3

As seen in previous seed soak experiments 3% hydrogen peroxide and 2% sodium carbonate treatments of 5, 15, and 30 minutes reduced the level of bacterial recovered from seed. Soaking seed in a solution of copper hydroxide and mancozeb was also effective. No treatment completely eliminated the presence of bacterial from seed.

Seed germination remained relatively constant, with the lowest number of seeds germinating in the 2% sodium carbonate 15 minute soak with 56% of seed germinating. The highest level of germination at 83% developed in seed soaked in 3% hydrogen peroxide for 15 minutes (Table 27).

Table 27. Effect of seed soaks for the control of *Pseudomonas*.

Treatment	Timing	Control achieved	Germination (%)
Hydrogen peroxide 3%	5 minutes	++	73.5
Hydrogen peroxide 3%	15 minutes	++	83
Hydrogen peroxide 3%	30 minutes	++	67.5
Sodium carbonate 2%	5 minutes	++	70
Sodium carbonate 2%	15 minutes	++	56
Sodium carbonate 2%	30 minutes	++	69
Copper hydroxide + mancozeb	5 minutes	+	73.5
Copper hydroxide + mancozeb	15 minutes	++	68
Sterile water	5 minutes	-	69
Sterile water	15 minutes	-	76.5
Sterile water	30 minutes	-	76
Un-inoculated Control Seed	-	-	68.5
Inoculated Control Seed	-	-	66.5
LSD (P=0.05)			ns

Where: - no reduction on fluorescing colonies, + minimal reduction, ++ high level of colony reduction +++ complete elimination of colonies.
Where: ns = there are no significant differences between treatments.

Experiment 4

Chlorine products had little effect on reducing bacterial numbers in treated seed. The most effective treatment was 2% chlorine dioxide for 5 minutes with solutions of hydrogen peroxide and peroxyacetic acid also reducing colony numbers compared to the remaining treatments which had no effect.

Seed germination was inhibited with the chlorine dioxide treatment as 46% of seed germinated compared to 66% in the control (Table 28).

Table 28. Effect of seed soaks for the control of *Pseudomonas*.

Treatment	Timing	Control achieved	Germination (%)
Chlorine dioxide 2%	5 minutes	++	46.5 c
Chlorine dioxide 2%	15 minutes	+	46 c
Hypochlorite 1000ppm	5 minutes	+	64.5 ab
Hypochlorite 1000ppm	15 minutes	-	63 ab
Sodium hydroxide 1000ppm	5 minutes	-	63.5 ab
Sodium hydroxide 1000ppm	15 minutes	-	53.5 bc
Hydrogen peroxide+ Peroxyacetic acid	5 minutes	+	65.5 ab
Hydrogen peroxide + Peroxyacetic acid	15 minutes	+	69.5 a
Sterile water	5 minutes	-	67 a
Sterile water	15 minutes	-	65.5 ab
Un-inoculated Control Seed	-	-	66.5 ab
Inoculated Control Seed	-	-	68 a
LSD (P=0.05)			13.37

Where: - no reduction on fluorescing colonies, + minimal reduction, ++ high level of colony reduction +++ complete elimination of colonies.
NB. Treatments with the same letter are not significantly different to one another.

Conclusion

These studies showed that *Pseudomonas syringae* pv. *porri* increases seedling mortality and establishment. Severe infections in mature plantings stain and rot stems resulting in unmarketable leeks.

Leek varieties Missile, Nova, Harpoon and Admiral were all susceptible to *Pseudomonas*. Preliminary fungicide screening indicated that copper sprays may reduce the infection levels however these fungicides need to be more extensively tested.

Seed borne infection of *Pseudomonas syringae* pv. *porri* was only eliminated with initial hot water treatments of 50^{o}C for 35 minutes and 55^{o}C for 25 minutes but with severe reductions in germination. The chemical soak treatments of 3% hydrogen peroxide, sodium carbonate and potassium permanganate reduced seed infection without reducing the germination. Further evaluation of seed treatments is required to locate a product that will eliminate the seed borne infection without compromising germination.

Leaf blight and Purple blotch

Leaf blight and purple blotch are two diseases causing cosmetic damage to leeks in Australia. Leaf blight is widespread in plantings throughout Australia while purple blotch was found in Victoria and Queensland. Experiments were carried out to determine the optimum temperature for growth of these fungi and to determine the effect of various fungicides on mycelial growth.

General materials and methods

Selective media: *Alternaria* and *Stemphylium* isolates were grown and maintained on V-8 agar (1). Isolates were stored in Muck soil (2) which comprised of a 1:1:1 mixture of UC soil: peat moss: vermiculite. This mixture was sterilised at 120^{o}C for 15 minutes, and re-sterilised after 12 hours. Isolates were stored in Muck soil and grown up on V-8 agar when required.

Isolates: 5 isolates of *Stemphylium* and *Alternaria* were collected and tested for pathogenicity. Pathogenic isolates were maintained for laboratory and greenhouse experiments in Muck soil and refrigerated at 4^{o}C. Isolates collected during the survey from various locations were used for laboratory experiments.

Table 29. *Stemphylium* and *Alternaria* isolates used for experimental work.

Isolate Number	Species	Location	Plant Part
619	*Alternaria porri*	Cranbourne, Victoria	Leaves
621	*Stemphylium botryosum*	Clyde, Victoria	Leaves
624 (a)	*Stemphylium botryosum*	Cranbourne, Victoria	Leaves
624 (b)	*Alternaria porri*	Cranbourne, Victoria	Leaves

Detection and isolation of fungi: *Stemphylium* and *Alternaria* isolates were collected from diseased tissue which had been put into humidity under UV light for 48 hours to induce sporulation. Conidia were removed and plated onto V-8 agar and plates were incubated at 22^{o}C in darkness for 7 days, placed under UV light for 24 hours to induce sporulation and then placed into darkness for a further 5 days.

Mycelial growth assessment: Fungal growth was measured as the mean of 2 radial dissects of the colonies and the percent inhibition determined relative to the growth of the control.

Inoculum: Muck soil infected with *Stemphylium* or *Alternaria* was placed onto V-8 agar and incubated at 22^{o}C for 7 days in darkness, plates were then placed under UV light for 24 hours to induce sporulation and re-incubated in the dark for a further 5 days. Conidia were re-isolated onto V-8 agar and incubated as previously mentioned. After this time conidia were collected from plates by adding 10ml of sterile water to each plate and removed from the agar with a sterile paint brush. The inoculum suspension was filtered through gauze and adjusted to the required spore concentration.

Chemical control: Four fungicides were evaluated for their control of *Stemphylium* and *Alternaria* (Table 30). The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Table 30. Chemicals used to determine their efficacy for the control of leaf blight and purple blotch.

Chemical	**Manufacturer**	**Active Ingredient**
Bravo plus®	Syngenta	500g/L chlorothalonil + 10g/L cyprocazole
Dithane®	Dow Agrosciences Aust	750g/Kg mancozeb
Score®	Syngenta	250g/L difenconazole
Rovral®	Bayer Cropscience Pty Ltd	250g/L iprodione

Laboratory experiments

In vitro

These studies were undertaken to determine the optimum temperature for the growth of *Stemphylium* and *Alternaria*, to determine whether different isolates were affected by temperature and the sensitivity of isolates to fungicides.

Objective: *To determine the optimum temperature for the growth of A. porri and S. botryosum.*

Materials and methods

Two *S. botryosum* (621 and 624 (a)) and two *A. porri* (619 and 624 (b)) isolates were used for this experiment. A 5mm plug of agar taken from the outer edge of a 10 day old *A. porri* or *S. botryosum* culture was plated onto petri plates containing TWA. Plates were incubated in the dark at 5, 10, 15, 20, 25, 30 and 35^{o}C and after 7 days the radial mycelial growth recorded on each of the 5 replicates for each temperature. The plates were then stored at room temperature (22^{o}C) for a further 7 days and assessed to determine if temperature inhibited the subsequent growth.

Results and Discussion

Stemphylium grew at all temperatures between 5 and 35^{o}C with the optimum temperature for both *Stemphylium* isolates being 25^{o}C. While not completely inhibited at any temperature tested, growth was reduced at 5, 10 and 35^{o}C. When held at room temperature for a further 7 days all isolates continued to grow, however the rate of mycelial growth on the 35^{o}C plates was not as great as that seen for the other temperatures (Figure 26 & 27).

The most favourable temperature for the growth of *Alternaria* was also 25^{o}C, with no temperature inhibiting mycelial growth completely. The lowest level of growth was observed at 5 and 35^{o}C and all isolates continued to grow at room temperature for a further 7 days (Figure 28 & 29).

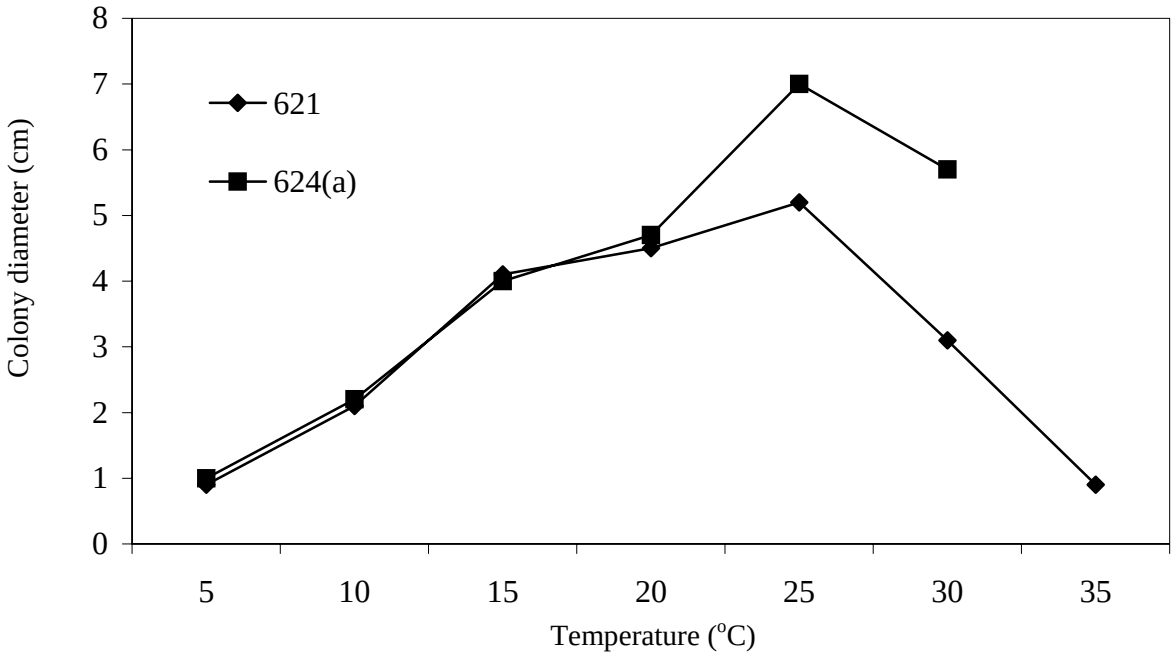


Figure 26. Effect of temperature on the mycelial growth of 2 isolates of *Stemphylium botryosum* after 7 days.

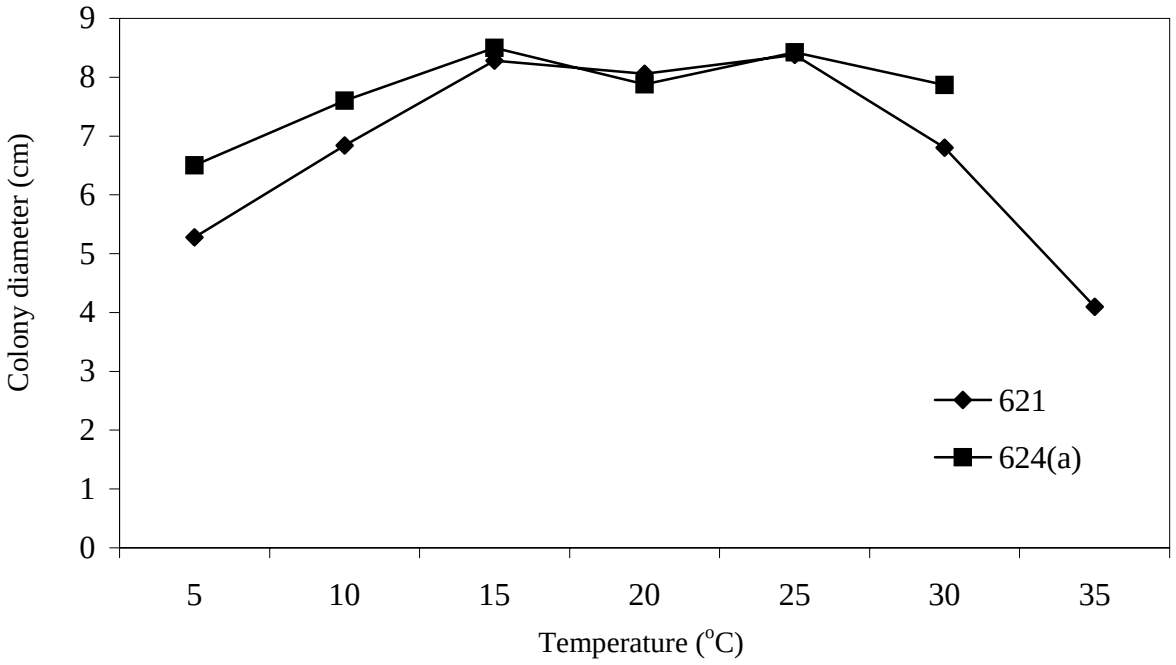


Figure 27. Effect of temperature on the mycelial growth of 2 isolates of *Stemphylium botryosum* incubated at each temperature for 7 days and re-measured after 7 days at room temperature.

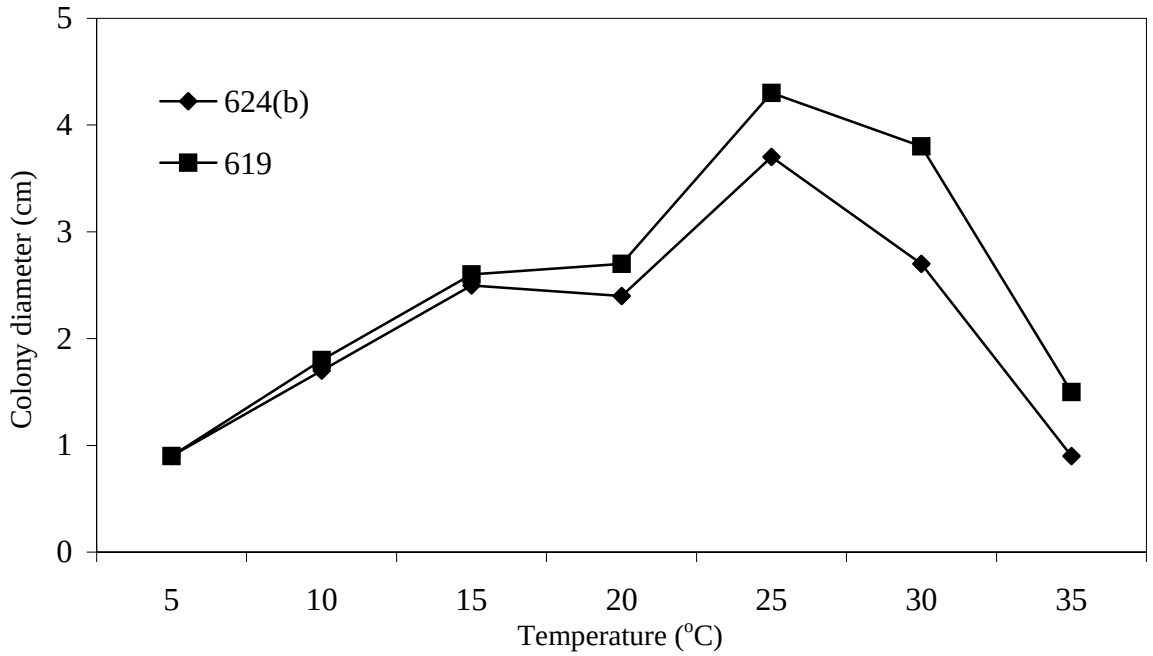


Figure 28. Effect of temperature on the mycelial growth of 2 isolates of *Alternaria porri* after 7 days.

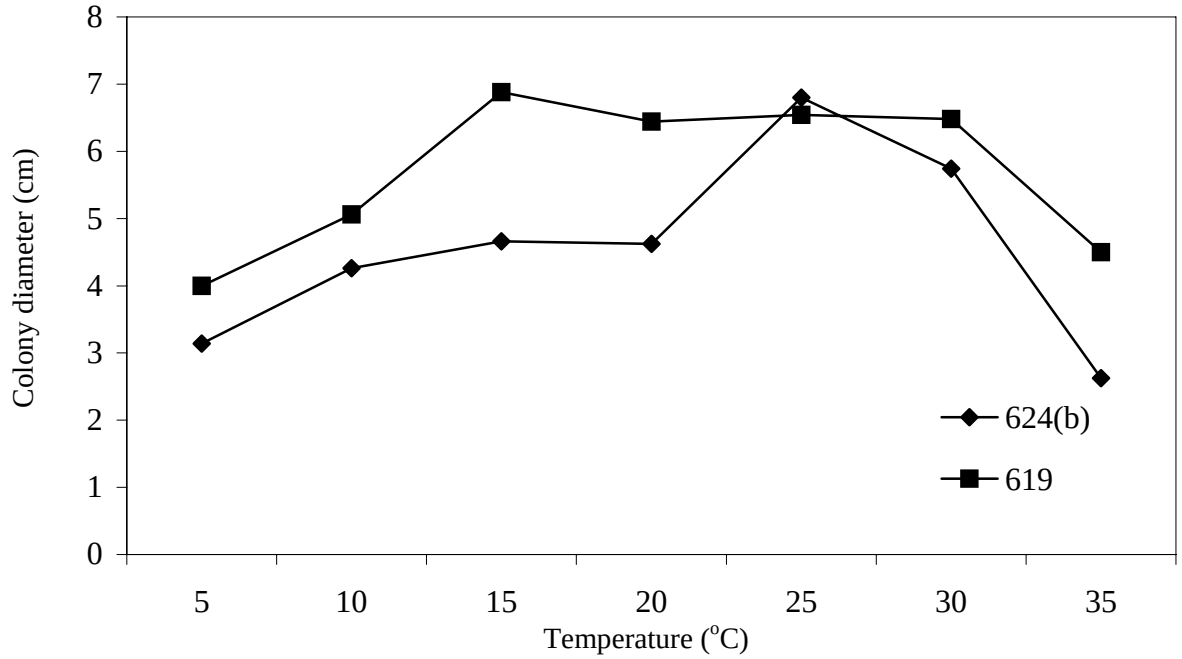


Figure 29. Effect of temperature on the mycelial growth of 2 isolates of *Alternaria porri* incubated at each temperature for 7 days and re-measured after 7 days at room temperature.

Objective: *To evaluate the sensitivity of S. botryosum and A. porri to fungicides.*

Materials and methods

PDA was amended with either chlorothalonil + cyprocazole, mancozeb, difenconazole or iprodione at the concentrations 0.01, 0.1, 1, 5, or 10ppm active ingredient. Fungicide suspensions were prepared as stock solutions from formulated products and added to agar just prior to pouring the plates at 55-60^{o}C. The plates were dried in a laminar flow cabinet for 30 minutes before a 5mm plug of agar taken from the outer edge of an actively growing *Stemphylium* (624(a)) or *Alternaria* (619) isolate was placed in the centre of 5 agar plates of each concentration. The plates were sealed with parafilm and incubated at 25^{o}C for 5 days and the radial diameter of mycelial growth measured. The sensitivity of the fungus was evaluated as mentioned in the general materials and methods.

Results and discussion

No fungicide completely inhibited the growth of *Stemphylium*. Difenconazole and iprodione at 10ppm inhibited the growth of *Stemphylium* by 76 and 78% respectively. Mancozeb was not inhibitory to the growth of *Stemphylium*. Chlorothalonil + cyprocazole had little inhibitory effect and at 5 and 10ppm the inhibition was below 20% (Figure 30).

The effect of difenconazole and iprodione on the growth of *Alternaria* was similar to that for *Stemphylium*. Both difenconazole and iprodione inhibited mycelial growth by 55 and 68% at 10ppm respectively. Chlorothalonil + cyprocazole and mancozeb had limited effect on reducing mycelial growth with 8-9% inhibition achieved at 10ppm (Figure 31).

Both difenconazole and iprodione are known to effectively control *Alternaria* diseases in many crops. While both should be effective on leeks, the diseases caused by the fungus are mainly cosmetic, and other management techniques apart from the use of fungicides should be encouraged. To be effective these fungicides would be applied every 10-14 days and this could mean up to 10-15 sprays are applied during the 5-7 month life of a leek crop.

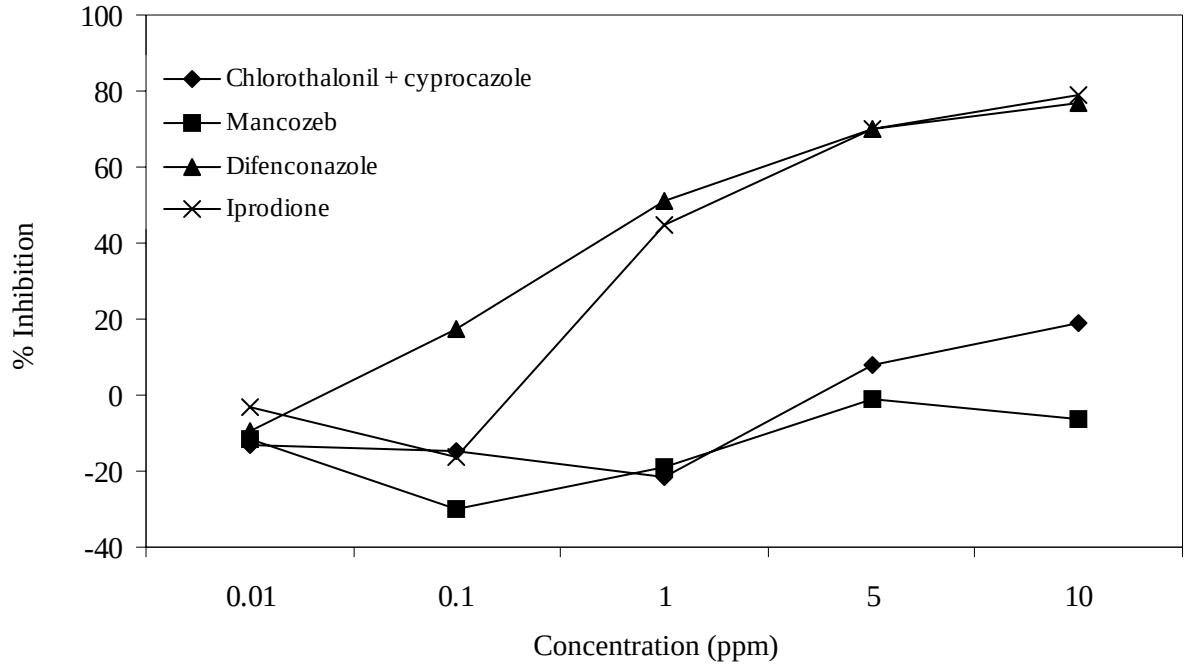


Figure 30. Effect of different concentrations of chlorothalonil + cyprocazole, mancozeb, difenconazole and iprodione on the mycelial growth of *S. botryosum* after 7 days.

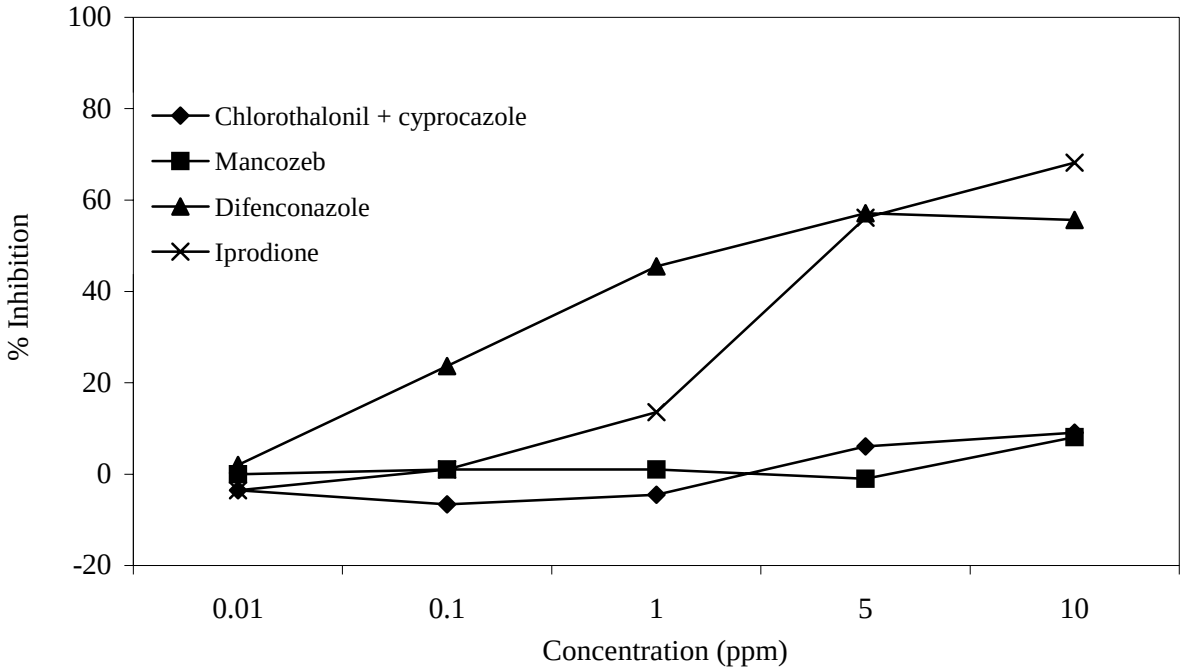


Figure 31. Effect of different concentrations of chlorothalonil + cyprocazole, mancozeb, difenconazole and iprodione on the mycelial growth of *A. porri* after 7 days.

OTHER PATHOGENS

Other pathogens found during the leek survey included *Pyrenochaeta terrestris*, *Cladosporium* sp. and *Colletotrichum circinans*. Experiments were undertaken to determine the sensitivity of these fungi to various fungicides. The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

General materials and methods

Isolates: All isolates were maintained on PDA and TWA and stored in culture on PDA at 4^{o}C. Isolates collected during the survey from various locations were used for laboratory experiments (Table 31).

Table 31. *Stemphylium* and *Alternaria* isolates used for experimental work.

Isolate Number	Species	Location	Plant Part
19	*Colletotrichum circinans*	Nairne, South Australia	Leaves
302	*Cladosporium* sp.	Lenswood, South Australia	Leaves
404	*Pyrenochaeta terrestris*	Wattleup, Western Australia	Roots
422	*Pyrenochaeta terrestris*	Wattleup, Western Australia	Roots
434	*Pyrenochaeta terrestris*	Carabooda, Western Australia	Roots

Detection and isolation of fungi: Leek crowns and roots were surface sterilised by soaking in 4% sodium hypochlorite solution for 3 minutes before rinsing thoroughly with RO water and dried in a laminar flow cabinet. 2mm sections of leek tissue from the diseased margin were removed and plated on TWA or PDA. Plates were sealed with parafilm and incubated at 22^{o}C with a 12 hour photoperiod for 7-10 days.

Mycelial growth assessment: Fungal growth was measured as the mean of 2 radial dissects of the colonies and the percent inhibition determined relative to the growth of the control.

Chemical control: Four fungicides were evaluated for their efficacy for the control of other pathogens found during the leek survey (Table 32). The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Table 32. Chemicals used to determine their efficacy for the control of *P. terrestris*, *Cladosporium* sp. and *C. circinans*.

Chemical	Manufacturer	Active Ingredient
Octave®	Bayer Cropscience	462g/L prochloraz
P-Pickel T®	Crop Care Australia	200g/L thiabendazole + 360g/Kg thiram
Dithane®	Dow Agrosciences Aust	750g/Kg mancozeb
Bravo Plus®	Syngenta	500g/L chlorothalonil + 10g/L cyprocazole

Laboratory experiments

In vitro

These studies were undertaken to determine the sensitivity of either *Pyrenochaeta terrestris*, *Colletotrichum circinans* or *Cladosporium* sp. to various chemicals. The fungicides used in these trials are for experimental purposes only and are not registered or permitted for use on leeks. The results generated in these trials are preliminary only, and further trials are required to confirm best management practices.

Objective: *To evaluate the sensitivities of P. terrestris to prochloraz or thiabendazole + thiram.*

Materials and methods

PDA was amended with either prochloraz or thiabendazole + thiram at the concentrations 0.01, 0.1, 1, 5, or 10ppm active ingredient. Fungicide suspensions were prepared as stock solutions from formulated products and added to agar just prior to pouring the plates at 55-60^{o}C. The plates were dried in a laminar flow cabinet for 30 minutes before a 5mm plug of agar taken from the outer edge of an actively growing *P. terrestris* isolates (404, 422 and 434) was placed in the centre of 5 agar plates of each concentration. The plates were sealed with parafilm and incubated at 25^{o}C for 5 days and the radial diameter of mycelial growth measured. The sensitivity of the fungus was evaluated as mentioned in the general materials and methods.

Results and discussion

Prochloraz had little inhibitory effect on the growth of *P. terrestris*. For example at 5 and 10ppm mycelial growth was reduced between 4-18% and 22-33% respectively (Figure 32).

Thiabendazole + thiram was inhibitory to the growth of *P. terrestris* with complete inhibition achieved at 5 and 10ppm (Figure 33).

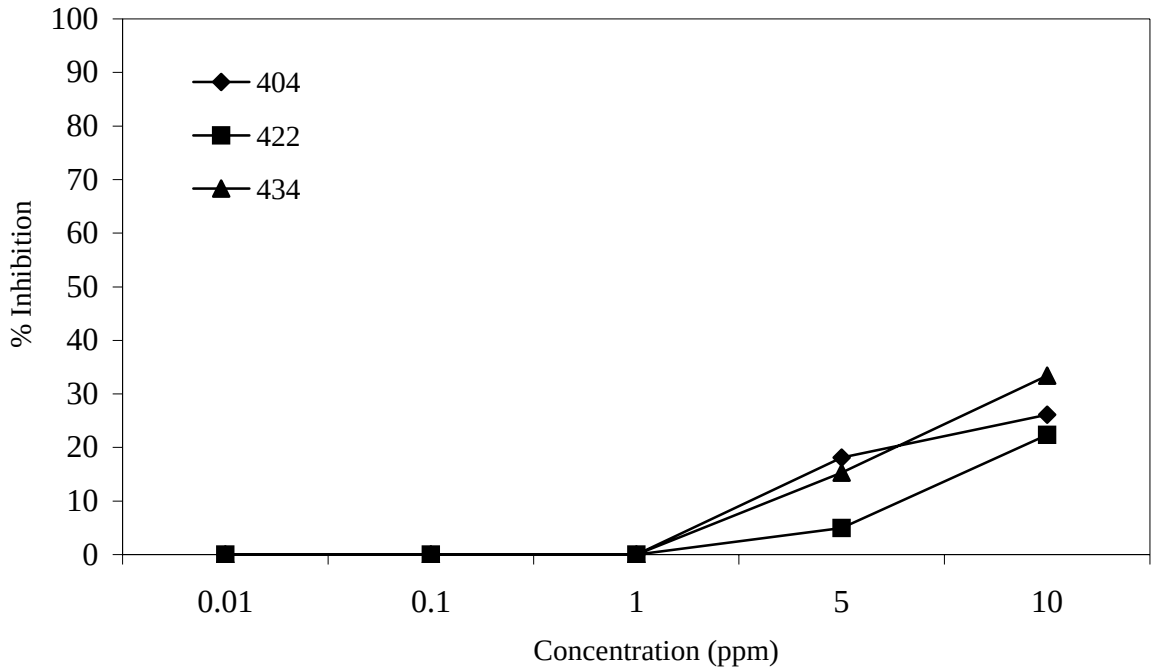


Figure 32. Effect of different concentrations of prochloraz on the mycelial growth of 3 *P. terrestris* isolates.

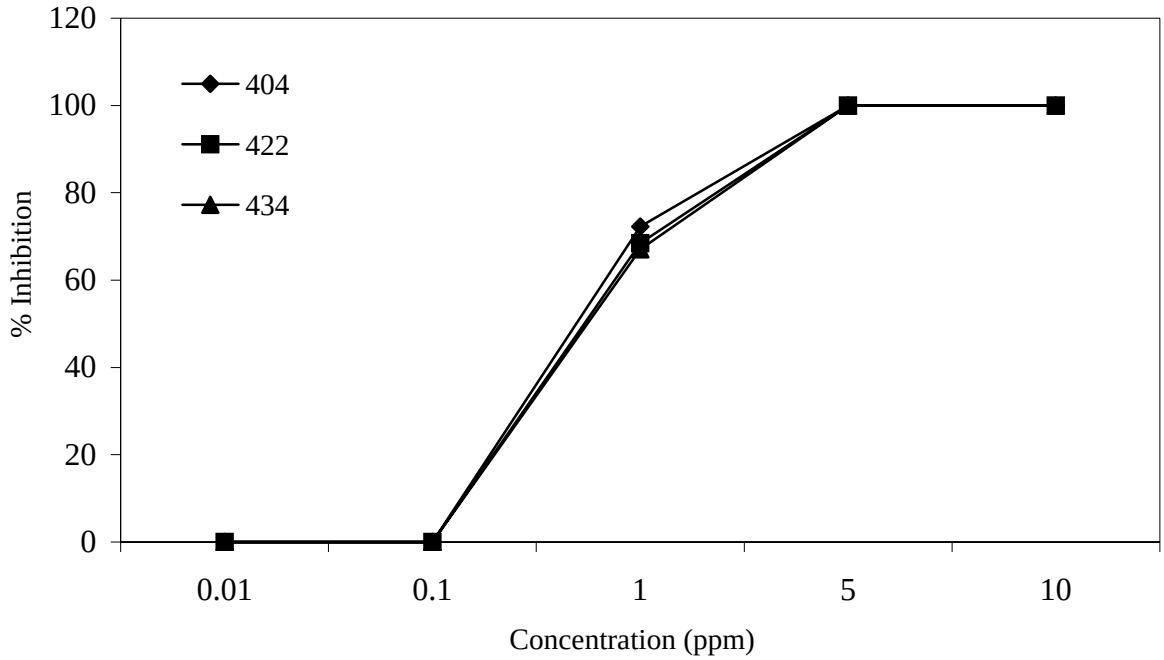


Figure 33. Effect of different concentrations of thiabendazole + thiram on the mycelial growth of 3 *P. terrestris* isolates.

Objective: *To evaluate the sensitivities of C. circinans, and Cladosporium sp. to fungicides in vitro.*

Materials and methods

PDA was amended with either chlorothalonil + cyprocazole or mancozeb at the concentrations 0.01, 0.1, 1, 5, or 10ppm active ingredient. Fungicide suspensions were prepared as stock solutions from formulated products and added to agar just prior to pouring the plates at 55-60^{o}C. The plates were dried in a laminar flow cabinet for 30 minutes before a 5mm plug of agar taken from the outer edge of an actively growing isolate of *C. circinans* (19) or *Cladosporium* (302) was placed in the centre of 5 agar plates of each concentration. The plates were sealed with parafilm and incubated at 25^{o}C for 5 days and the radial diameter of mycelial growth measured. The sensitivity of the fungus was evaluated as previously mentioned in the general materials and methods.

Results and discussion

Mancozeb at 5 and 10ppm completely inhibited mycelial growth of *Colletotrichum*. Complete inhibition was not achieved with chlorothalonil + cyprocazole with the most effective concentration 10ppm reducing growth by 72% (Figure 34).

Complete inhibition of *Cladosporium* mycelial growth was not achieved at any concentration tested, however mancozeb at 10ppm inhibited growth by 83% and chlorothalonil + cyprocazole at 10ppm by 59% (Figure 35).

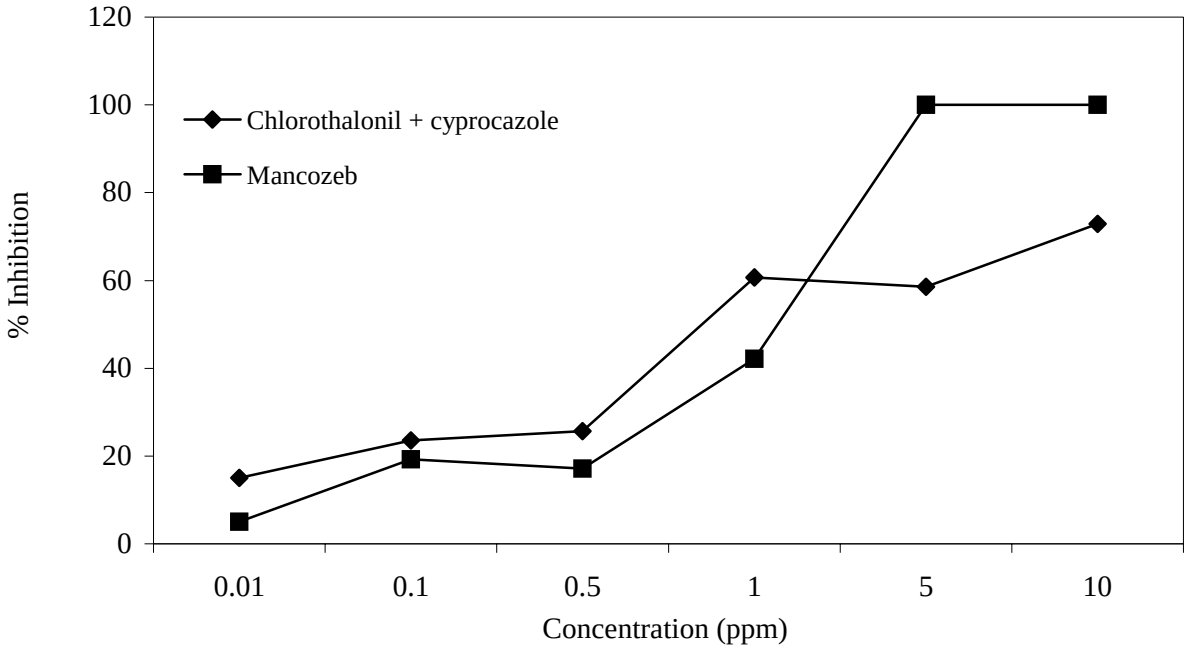


Figure 34. Effect of different concentrations of chlorothalonil + cyprocazole, and mancozeb on the mycelial growth of *C. circinans*.

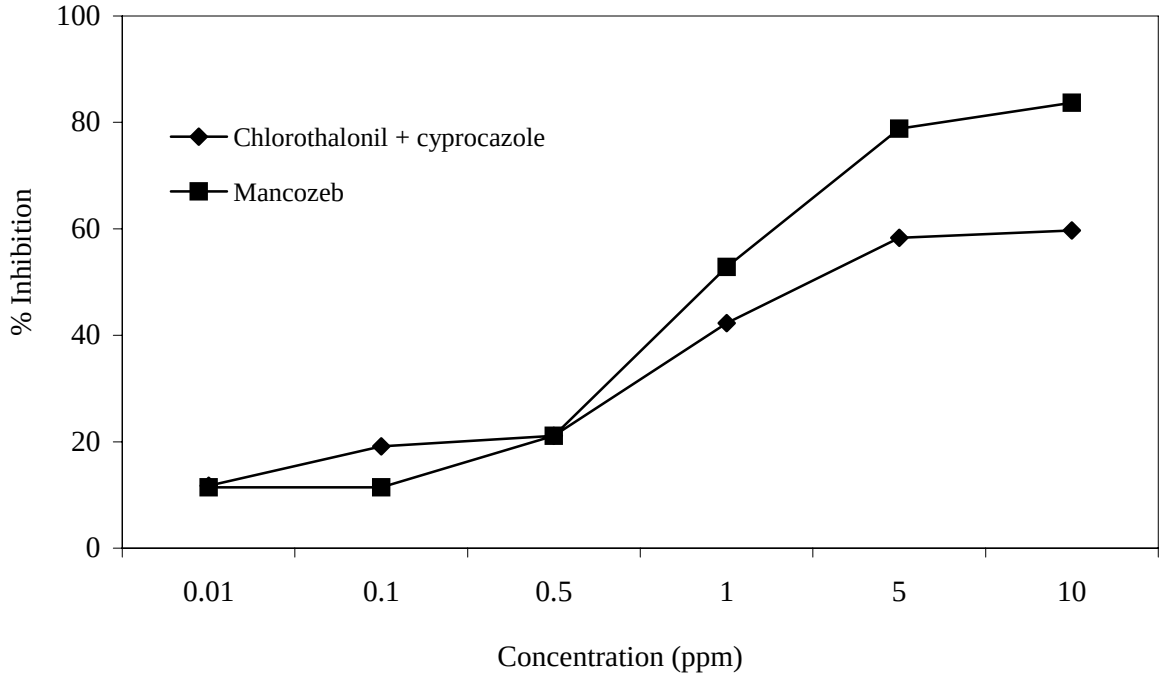


Figure 35. Effect of different concentrations of chlorothalonil + cyprocazole, and mancozeb on the mycelial growth of *Cladosporium*.

Conclusion

Initial evaluation of fungicides for the control of minor pathogens of leeks provided some promising results. Thiabendazole + thiram completely inhibited the growth of *P. terrestris* while prochloraz and mancozeb inhibited the growth of *Cladosporium* and *Colletotrichum* with complete inhibition of *Colletotrichum* mycelial growth achieved with mancozeb.

These pathogens were recorded on leeks during the project survey however damage to leeks from these pathogens is minor, causing minimal economic losses. Therefore no further work was undertaken.

TECHNOLOGY TRANSFER

These results of this research have been presented direct to growers, nurseries and industry representatives. In addition many articles have been published in Good Fruit and Vegetable, the Grower and WA Grower magazines as well as the production of newsletters.

Dr Trevor Wicks attended the Allium conference in Georgia, USA during November 2000 and subsequently visited researchers in the USA, England and the Netherlands and Mrs Barbara Hall visited growers in Queensland, June 2002. The information gained from these overseas sources was presented to growers in several meetings as well as in the HAL overseas travel report.

Initial Survey Property Visits

- 2000-2003 – Nairne, Murray Bridge and Langhorne Creek, South Australia.
- July 2001 – Clyde and Cranbourne, Victoria.
- July 2001 – Boomeroo Nursery, Victoria.
- April 2002 –Wanaroo and Wattleup, Western Australia.
- June 2002 – Stanthorpe, Queensland.
- November 2000 – Georgia, USA.

Workshops

- Growers workshop Cranbourne (Vic) October 2004.
- Grower meeting Virginia Horticulture Centre (SA) April 2004 (as part of the National Vegetable Working Pathologists meeting)
- Growers workshop Cranbourne (Vic) February 2003.

Articles

- Victorian leek growers get update on diseases. Good Fruit & Vegetables(2004) December, pp 19.
- Newsletter – Issue 3 October 2004.
- Leek diseases: two major concerns. Good Fruit & Vegetables (2004) July, pp 21.
- Newsletter – issue 2 February 2003.
- Leek diseases confirmed by SARDI study. The Grower (2002) September, pp 38.
- Leek disease project spots rot. The Grower (2001) March pp. 32.
- Diseases of leeks in Southern Australia. Good Fruit & Vegetables (2002) September.
- Newsletter - Issue 1 August 2002.

Conference presentations

- Diseases and Disorders of Leeks (*Allium porrum* L.) in Southern Australia. (Poster) 8th International Congress of Plant Pathology in Christchurch, New Zealand, incorporating the 14th Australasian Plant Pathology Conference (2003) February.

- Bacterial Diseases of Leeks in the Southern Hemisphere. (Workshop presentation) 8th International Congress of Plant Pathology in Christchurch, New Zealand, incorporating the 14th Australasian Plant Pathology Conference (2003) February.

- Pathogenicity testing of Fusarium foot rot in leeks. (Poster) 3rd Australasian Soilborne Diseases Symposium. (2004) February.

RECOMMENDATIONS

This project has shown that Fusarium foot rot and bacterial blight are the two most economically important diseases of leeks in Australia.

Studies need to be done to determine the level of these diseases on commercial seed used in Australia and in particular if any area of seed production or cultivar has high levels of infection.

Seed treatments need to be further evaluated to determine the efficacy of physical and chemical treatments in eliminating pathogens from seed.

Further testing also needs to be done on the use of chemical and biological treatments to reduce or prevent plants becoming infected with *Fusarium* in the nursery and after planting in the field.

Studies should also continue to evaluate fungicides and spray programs for the control of *Alternaria* and *Stemphylium*, as these two pathogens may become more important in the future particularly if there is climate change.

Studies need to be carried out to evaluate seed treatments for the control of bacterial infection in seed, and whether these treatments effect germination.

The spread of bacterial blight in plantings needs to be evaluated and whether different copper formulations can reduce spread and infection levels.

As a results of these studies growers should:

1) Use disease free seed - from a known source and that has been treated and properly certified as disease free.

2) Ensure that seedlings are grown in pasteurised or treated soil.

3) Ensure that seedlings are grown in a “clean” environment.

4) Obtain seedlings from a reputable source.

5) Avoid trimming the leaves of seedlings or other operations in the nursery that could spread bacteria through seedling batches.

6) Drench seedlings with a suspension of prochloraz or carbendazim to reduce the likelihood of soil infection with *Fusarium*.

7) Avoid injuring seedlings at planting.

8) Fungigate seedlings with prochloraz or carbendazim after planting.

9) Avoid stressing seedlings within 4 to 8 weeks after planting.

NB. The products prochloraz and carbendazim are currently not registered or permitted for use on leeks.

ACKNOWLEDGMENTS

We wish to thank the leek growers of Australia and related industry personnel for participating and being involved in the surveys. The leek growers in South Australia and Victoria for their co-operation and help in allowing field trials to be conducted on their properties. A special thank you to the leek growers of South Australia for allowing the extensive surveying of plantings and providing seedlings, especially John Cranwell for all his time, effort and guidance during the course of the project.

We also gratefully acknowledge Horticulture Australia Limited, Australian government and the Vegetable industry for financially supporting this research.

All the technical staff at the Plant Research Centre and the Lenswood Research Centre for their help in undertaking this project.

We wish to thank Michael Priest, Dr Ric Cother and Dorothy Noble from the New South Wales DPI for identifying leek pathogens throughout the project.

DISCLAIMER

The results in this report are submitted on the basis that the tests were conducted by SARDI in accordance with the protocol requested by of agreed to by your company, and using test chemicals based on scientific information currently available to SARDI.

Neither SARDI nor its officers accept any liability resulting from this interpretation or use of the information contained herein. Use of information is at the risk of the user to the extent permissible by law.

REFERENCES

1. Dhingra, O.D., and Sinclair, J.B. (1985). Basic Plant Pathology Methods. CRC Press, Inc., Boca Raton, FL pp 355.

2. Everts, K.L., and Lacy, M.L. (1990). The influence of Dew Duration, Relative Humidity, and Leaf Senescence on Conidial Formation and Infection of Onion by *Alternaria porri*. Phytopathology Vol 80, No. 11: 1203-1207.

3. Fahy, P.C. and Persley, G.J. (1983). Plant Bacterial Diseases – A diagnostics Guide.

4. Jennings, D.M., Ford-Lloyd, B.V. and Butler, G.M. (1990). Effect of plant age, leaf position and leaf segment on infection of leek by leek rust, *Puccinia allii*. Plant Pathology 39:591-597.

5. Jordan, M.M., Maude, R.B. and Burchill, R.T. (1990) Sources, survival and transmission of *Cladosporium allii* and *C. allii-cepae*, leaf blotch pathogens of leek and onion. Plant Pathology 39:237-241.

6. Koike, S.T. and Henderson, D.H. (1998). Purple botch caused by *Alternaria porri*, on leek transplants in California. Plant Diseases 82 (6): 710.

7. Koike, S.T., Henderson, D.M., Barak, J.D. and Gilbertson, R.L. (1997). A leaf bight disease of leek in California caused by *Pseudomonas syringae*, and association of the pathogen with seed. Phytopathology 87: S53. Publication no P-1997-0380-AMA.

8. Kioke, S.T., BarakJ.D., Handerson, D.M., and Gilbertson, R.L. (1999). Bacterial blight of leek: A new disease in California caused by *Pseudomonas syringae.* Plant Disease 83:165-170.

9. Lot, H., Chovelon, V., Souche, S. and Delecolle, B. (1998). Effects of Onion Yellow Dwarf and Leek Yellow Stripe viruses on symptomatology and yield loss of three French garlic cultivars. Plant Disease 82 (12):1381-1385.

10. Smilde, W.D., van Nes, M. and Reinink, K. (1995). Resistance to *Phytophthora porri* in leek and some of its wild relatives. Euphytica 83:131-138.

11. Smith, B.M. and Crowther, T.C. (1992). Field assessments of the reaction of leek cultivars to leek rust. Ann. Appl. Biol. 120 (Supplement).

12. Suheri, H and Price T.V. (2000). Stemphylium leaf blight of garlic (*Allium sativum*) in Australia. Australasian Plant Pathology 29:192-199.

13. Van Duk, P. (1993). Survey and characterisation of potyviruses and their strains of Allium species. Neth. J. Pl. Path. 99, Supplement 2:1-48.

14. Schaad, N.W. (1988). Laboratory Guide for the Identification of Plant Pathogenic Bacteria. 2nd Edition.

15. Burgess, L.W., Summerell, B.A., Bullock, S., Gott, K.P. and Backhouse, D. (1994). Laboratory Manual for Fusarium Research (3rd Edition).