Managing diseases of leeks

Catherine Hitch
SA Research & Development Institute

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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
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.

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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.
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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 seedling drenches 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°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°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°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°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°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°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°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°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 $3.7 \times 10^5$ (*Stemphylium*) and $6.5 \times 10^4$ (*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.

<table>
<thead>
<tr>
<th></th>
<th>South Australia</th>
<th>Victoria</th>
<th>Western Australia</th>
<th>Queensland</th>
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<td><strong>Stemphylium</strong></td>
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<td><strong>Alternaria</strong></td>
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<td><strong>Colletotrichum</strong></td>
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<td><strong>Botrytis</strong></td>
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<tr>
<td><strong>Pyrenochaeta</strong></td>
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<td><strong>Cladosporium</strong></td>
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<td><strong>Viruses</strong></td>
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<td><strong>Nematodes</strong></td>
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</table>

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.
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.
- April 2002 – Wanaroo and Wattleup, Western Australia.
- June 2002 – Stanthorpe, Queensland.
- November 2000 – Georgia, USA.

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

Articles
- Newsletter - Issue 1 August 2002.

Conference presentations
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 result 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

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