HAL

Know-how for Horticulture™

Integrated management of Pythium diseases of carrots

E Davison and A McKay
Department of Agriculture,
Western Australia

Project Number: VG98011

VG98011

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 0333 6

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

Horticulture Australia

FINAL REPORT

HORTICULTURE AUSTRALIA PROJECT VG98011

INTEGRATED MANAGEMENT OF *PYTHIUM* DISEASES OF CARROTS

E. M. Davison and A. G. McKay
Department of Agriculture, Western Australia

DEPARTMENT OF Agriculture

THE GOVERNMENT OF WESTERN AUSTRALIA

Horticulture Australia

September 2001

HORTICULTURE AUSTRALIA PROJECT VG 98011

Principal Investigator

Elaine Davison
Research Officer, Crop Improvement Institute,
Department of Agriculture,
Locked Bag 4, Bentley Deliver Centre,
Western Australia 6983
Australia

Email: edavison@agric.wa.gov.au

This is the final report of project VG 98011 Integrated management of *Pythium* diseases of carrots. It covers research into the cause(s) of cavity spot and related diseases in carrot production areas in Australia, together with information on integrated disease control.

The project was funded by Horticulture Australia and the Department of Agriculture, Western Australia.

Contents

Industry summary

Background

Carrots are the third most important vegetable produced in Australia with a gross value of production of $ 170 million per annum. In eastern Australia they are primarily grown for domestic consumption, while production in Western Australia is focused on the export trade.

Cavity spot is a serious disease of carrots that has the potential to severely reduce marketable yield. A survey of Western Australian carrot crops, conducted as part of HRDC Project VG036, showed that cavity spot was present in almost half of 200 crops, and resulted in a 10 per cent or greater marketable yield loss in 16 per cent of these crops.

World wide, cavity spot is caused by two species of *Pythium, P. violae* and *P. sulcatum*. These are soil-borne fungi that build up on sites that have been repeatedly cropped to carrots. *P. sulcatum* was identified as the cause of cavity spot in Western Australia in the HRDC Project VG 95010. Other *Pythium* diseases of carrots include damping off, resulting in low root numbers at harvest, and root dieback, resulting in forked and misshapen carrots. Thus there are a number of *Pythium* diseases that affect carrots at different times during their development.

Part of HRDC Project VG 95010 investigated ways of controlling cavity spot and other *Pythium* diseases on Western Australian carrot farms in order to develop a system of integrated disease management for local growers. The control measures investigated included chemical control and control using tolerant cultivars. This research programme was greatly aided by the development of a cavity spot disease nursery site at the Medina Research Station that was used for variety trials and host range studies.

Project aims

The aims of this project were to build on the integrated disease management work from HRDC Project VG 95010. There were three aims:

1. To extend the work to other states to determine whether what had been achieved in Western Australia was directly applicable to carrot production in southern and eastern Australia. Specifically, to determine whether *Pythium sulcatum* is the cause of cavity spot elsewhere in Australia.
2. To complete the research undertaken as part of HRDC Project VG 95010.
3. To extend this work to industry.

Major findings

1. Survey of *Pythium* associated with cavity spot and related carrot diseases.

Colleagues in other states forwarded *Pythium* isolates from carrots to Western Australia for identification and pathogenicity testing. *P. sulcatum* was the most widespread pathogen, occurring in all states, and from most carrot growing regions. *P. violae* was also isolated, but only from carrot farms in the River Murray basin. This is the first record of *P. violae* from carrots in Australia.

If *P. sulcatum* has been introduced with the carrot industry we would expect it to be genetically uniform, but if it was present on sites before carrots were grown, then we

would expect it to be quite variable. DNA analysis of *P. sulcatum* isolates showed that it was quite variable, with Queensland isolates clustering separately from those in Tasmania and Western Australia. This diversity suggests that it is a cosmopolitan species that may have been present on sites before carrots were grown.

A small survey of carrot crops in Victoria and South Australia showed that the incidence of cavity spot varied from 0 to 79 per cent, and was more common in Victoria than South Australia.

2. Integrated disease control: cultural methods

Additional work on the host range of *P. sulcatum* confirmed that it is restricted to the carrot family. The cereals that are used for seedling wind protection are not infected.

A rotation trial was set up on a severely infested site, in which carrots were grown in rotation with the non-host broccoli. Carrots were seeded after one, two or three broccoli crops. Seedling infection was less when carrots followed broccoli, and this was reflected in a reduction in forking and an increase in root length in the harvested crop. There was a decrease in the incidence and severity of cavity spot in two of the three harvests, but the results were inconsistent. This experiment showed that *P. sulcatum* is able to survive for at least 21 months in the absence of a host.

Solarisation has the potential to control soil-borne diseases through passive solar heating. We found that it has the potential to control cavity spot caused by *P. violae* because *P. violae* did not survive 2 hr. at 35 ^{0}C. It is unlikely to control *P. sulcatum* however, because it survived for 2 hr. at 45^{0}C.

3. Integrated disease control: chemicals

HRDC Project VG 95010 showed that the reason why the fungicide metalaxyl did not always control cavity spot was the result of poor persistence in soil (probably the result of enhanced microbial degradation), not the development of tolerance in *P. sulcatum.*

A survey was carried out as part of VG 98011 to determine whether enhanced breakdown of metalaxyl was a widespread problem on carrot farms. The results indicate that it is a problem on some farms.

The ability of other chemicals and microbial formulations to control cavity spot disease was tested on an infested site at the Medina Research Station. None of the products used significantly reduced the level of cavity spot, although Amistar® is worthy of further work.

4. Integrated disease control: tolerant varieties

Variety screening for cavity spot tolerance has continued in the disease nursery at the Medina Research Station in Western Australia. In addition, three on-farm trials were seeded in Victoria. Many of the most cavity spot tolerant varieties did not produce the high root quality demanded by the export market. Stefano, however, combines moderate yield, cavity spot tolerance and high root quality, and has become the industry stand variety throughout Australia.

Technical summary

The cause of cavity spot and other *Pythium* diseases of carrots in Australia

Cavity spot disease of carrots is caused by *Pythium* spp. *P. violae* and *P. sulcatum* are the most important causes of cavity spot worldwide. *P. sulcatum,* but not *P. violae*, causes this disease in Western Australia. A survey of *Pythium* spp. associated with carrot crops in eastern and southern Australia showed that *P. sulcatum* was the most widespread pathogenic species, occurring in all states and isolated from most regions. *P. violae* was recovered from two regions, one in Victoria and one in South Australia, but both in the River Murray basin. This is the first record of *P. violae* from carrots in Australia

The diversity of *P. sulcatum* isolates, as shown by DNA analysis, suggests that it is a cosmopolitan species that may occur on native Australian Apiaceae.

A small survey of carrot crops in Victoria and South Australia showed that the incidence of cavity spot varied from 0 to 79 per cent, and was more common in the crops from Victoria than those from South Australia.

Cultural methods for controlling cavity spot and other *Pythium* diseases

Host range and rotation

The major hosts of *Pythium sulcatum* are members of the carrot family (Apiaceae). Grasses (barley, maize, oats, rye and wheat) used for wind protection, and un-related vegetables, are not infected.

In an experiment on a badly infested site at the Medina Research Station, carrots were planted after one, two or three broccoli crops. There was a significant reduction in the incidence and severity of seedling infection by *P. sulcatum* when carrots followed broccoli. At harvest this was associated with decreased forking and increased root length, resulting in an increase in export yield. There was a decrease in the incidence and severity of cavity spot in two of the three plantings where carrots followed broccoli, but these results were inconsistent. Oospores of *P. sulcatum* are able to survive for at least 21 months in the absence of a host.

Solarisation

Solarisation is a cultural method for controlling soil-borne diseases where soil is heated by solar energy. Its potential to reduce cavity spot was assessed in experiments that determined the survival of *P. sulcatum* and *P. violae* at elevated temperatures Isolates of *P. violae* failed to survive for 2 hr. at 35^{0}C while *P. sulcatum* survived for 2 hr. at 45^{0}C, and 6 hr. at 42.5^{0}C. In the field it is unlikely that temperatures achieved by solarisation will be high enough to reduce the inoculum potential of *P. sulcatum,* although these temperatures may be sufficient to reduce the inoculum of *P. violae.*

Chemical methods for controlling cavity spot and other *Pythium* diseases

In a field experiment on a badly infested site, cavity spot control was attempted with a number of commercially available chemical and microbial formulations. Seedling harvests showed that *Pythium* infection was only reduced in the metalaxyl treatment. At the final harvest there was no significant reduction in the incidence or severity of cavity spot in any treatment although Amistar® is worthy of further work.

A survey was carried out to determine whether there was evidence of enhanced breakdown of the fungicide metalaxyl on sites where it has been used in the past. Metalaxyl was added to soil samples from carrot properties in South Australia, Tasmania and Western Australia and the half-life determined by chemical analysis. The half-life varied from less than 1 day to 43 days, compared with a published value of 70 days. Enhanced breakdown of metalaxyl appears to be a widespread problem.

Varietal tolerance to cavity spot

Identification of carrot varieties tolerant to cavity spot, that are also suitable for export production, is an important part of integrated disease control. Between 1999 and 2001 further variety screening was carried out in a cavity spot disease nursery at the Medina Research Station in Western Australia. Three farm trials were also planted in Victoria to confirm the relative cavity spot tolerance of varieties. Many of the most cavity spot tolerant varieties identified did not produce the high root quality demanded by export markets. The variety Stefano combines the characters of moderate yield and cavity spot tolerance with high root quality. Stefano has become established as the industry standard variety throughout Australia.

Technology transfer

- As a lead-in to VG 98011, Dr. Geoff White, Horticultural Research International, U.K., visited Australia in October 1998. This visit was a direct result of collaboration on project VG 95010. Whilst in Australia he reviewed the cavity spot research programme and discussed future collaboration.
- Dr. White and Dr. Davison presented a seminar focusing on integrated management of cavity spot to carrot growers on 16th October 1998, and visited carrot growers with Mr. McKay to discuss aspects of cavity spot control.
- Dr. White, Dr. Davison and Mr. McKay visited carrot-growing areas along the River Murray in South Australia and Victoria on 19th-20th October 1998, with Dr. Trevor Wicks (SARDI, Adelaide), Ms. Shirley Sylvia (SARDI, Loxton), Ms Sally-Ann Henderson (Agriculture Victoria, Mildura) and Dr. Bronwyn Wiseman (Agriculture Victoria, Knoxfield). The visits were made to consolidate collaborative arrangements for VG 98011.
- Dr. White, Dr. Davison and Mr. McKay visited carrot-exporting companies near Devonport, Tasmania, with Dr. Hoong Pung (Serve-Ag Research, Devonport). They presented a seminar to carrot producers and researchers on 21st October 1998. Radio interviews about project work were conducted with ABC regional radio.
- Dr. Davison visited carrot-growing properties in the Fassifern Valley with Mr. Rob. O'Brien (Queensland Department of Primary Industry, Indooroopilly) on 3rd November 1998. This visit consolidated collaborative arrangements for VG98011.
- Dr. Davison visited Dr. White (HRI) on 5th February 1999 to further the informal collaboration on cavity spot research.
- Dr. Davison visited Dr. Peter Gladders (ADAS, UK) and Dr. Peter Wright (Watton Produce, UK) to discuss cavity spot control in Europe.
- An article on cavity spot disease, 'Research to put an end to trouble spot', that included results of the variety trials, was published in the April 15, 1999, issue of Farm Weekly.
- Updated cavity spot control recommendations summarised in a colour Farmnote (Davison, E. and McKay, A. 1999. Cavity spot of carrots. Agriculture Western Australia Farmnote 29/99).
- Carrot webpage established in 1999 at: www.agric.wa.gov.au/programs/hort/Carrots
- Allan McKay and Elaine Davison (1998/99). Carrot export growth depends on keeping cavity spot under control. *Journal of Agriculture, Western Australia,* **40,** 19-23. This article focuses on the variety trials that have been undertaken at the Medina Research Station.
- Davison, E. M. and McKay, A. G. (1999). Reduced persistence of metalaxyl in soil associated with its failure to control cavity spot of carrots. *Plant Pathology,* **48,** 830-835.
- Poster paper 'Failure to control *Pythium* is associated with reduced persistence in soil' by E. M. Davison and A. G. McKay, presented at 12th Biennial Conference of the Australasian Plant Pathology Society in Canberra 27th to 30th September 1999.

- Western Australian carrot growers given an update on VG 98011 at a Research and Development Forum for the WA carrot industry, conducted by Sally-Ann Henderson, on 15th October 1999.
- The results of the *Pythium* survey and variety screening were presented to Western Australian growers on a Field Walk at the Medina Research Station on 14th April 2000.
- Dr. Davison and Mr. McKay visited New Zealand from 29th April to 5th May 2000, at the invitation of colleagues from Crop & Food Research. They presented three workshops on field and post harvest diseases of carrots to growers in the major carrot growing areas of Pukekohe, Ohakune and Lincoln. This was an opportunity to strengthen links between carrot researchers in Australia and New Zealand.
- Dr. Davison presented a seminar to Environmental Biology students at Curtin University of Technology, Perth, on 'Why don't chemicals always control plant diseases?' on 23rd August 2000.
- Carrot Conference Australia, 25th to 27th October 2000, provided an excellent opportunity to present the results of VG98011 to growers, researchers and industry representatives. Two talks were presented: 'Carrot variety tolerance to cavity spot' by A. G. McKay and E. M. Davison, and 'Cavity spot in Australia' by E. M. Davison and A. G. McKay. The conference delegates visited the Medina Research Station to view trials in the ground.
- Past and present work on cavity spot was presented immediately after Carrot Conference Australia at a Cavity Spot Technical Workshop, on 28th October 2000 at Technology Park, Bentley. About 50 people from Australia and overseas attended this workshop. Collaborators on VG 98011 from Queensland, Victoria, Tasmania and South Australia attended this workshop. They contributed by reviewing the carrot industry and the importance of cavity spot disease in their respective states. Dr. Davison and Mr. McKay presented talks on '*Pythium* in Western Australian carrots – the early years', '*Pythium* isolates from Australian carrots', 'Lime reduces cavity spot', 'Chemical control and the problem of enhanced biodegradation' and 'Host range and rotation'.
- Dr. Davison and Mr. McKay visited carrot-growing regions of Victoria from 5th to 7th February 2001. They assisted Ms Robyn Brett (AgVictoria, Knoxfield) harvest cavity spot screening trials on growers' properties at Dandenong and Boneo, and discussed trial results with these growers. They also visited carrot-growers in the Robinvale region with Ms. Sally-Ann Henderson (AgVictoria, Mildura).
- Dr. Davison presented a seminar on '*Pythium* diseases of carrots in Australia' at HRI, Wellesbourne, UK, on 26th April 2001.
- Results of chemical trials, the rotation trial and variety screening were presented to Western Australian growers at a Carrot Field Walk at the Medina Research Station on 15th June 2001.
- Elaine Davison and Allan McKay (2001). Advancing carrot disease control. *Good Fruit & Vegetables*, **12** (2), 27-29.
- Elaine Davison and Allan McKay (2001). Managing cavity spot in Australia. *Carrot Country* **9** (3), 8-10.

Section 1. Identification of *Pythium* spp. from carrots throughout Australia

E. M. Davison, G. MacNish[1], P. A. Murphy, A. G. McKay, R. Brett[2], R. Cole[3], S-A. Henderson[4], H. Pung[5], R. O'Brien[6] and L. Tesoriero[7]

1.1 Identification of *Pythium* spp. from carrots throughout Australia (99pe2)

Summary *Pythium violae* and *P. sulcatum* are the most important causes of cavity spot disease of carrots worldwide. *P. sulcatum,* but not *P. violae*, is associated with this disease in Western Australia. A survey was conducted to determine whether *P. sulcatum* was the main pathogenic species associated with cavity spot and other *Pythium* diseases, in carrot growing regions of Eastern Australia. *Pythium* isolates from carrots or carrot sites in Queensland, New South Wales, Victoria, South Australia and Tasmania were identified by morphological means and used in *in vitro* pathogenicity tests. Sixty-six per cent of the 213 isolates identified were grouped into three taxa that were consistently pathogenic. *P. sulcatum* (91 isolates) was the most widespread pathogenic species, occurring in all states and isolated from most regions. *P. violae* (11 isolates) was recovered from two regions, one in Victoria and one in South Australia, but both in the River Murray basin. The third group, slow growing isolates that formed filamentous sporangia but no oogonia (39 isolates), were shown to be *P. sulcatum* by RE-PCR analysis.

This is the first record of *P. violae* from carrots in Australia.

Introduction

Carrots are an important horticultural crop in Australia. Cavity spot is one of the most important diseases of carrots worldwide. It is caused by *Pythium* spp., notably *P. violae* and *P. sulcatum* (White, 1986; Nagai *et al.,* 1986; Montfort and Rouxel, 1988; Vivoda *et al.*, 1991; Breton and Rouxel, 1993; Benard and Punja, 1995). In Western Australia (WA) cavity spot is associated with *P. sulcatum* (Davison and McKay, 1998), but it is not known which species are associated with this disease in other states of Australia.

P. violae and *P. sulcatum* differ in their host range (Kalu *et al.,* 1976; Schrandt *et al.*, 1994; McKay and Davison, 2000) and metalaxyl sensitivity (White *et al.,* 1988; Breton and Rouxel, 1993). Thus it is important that *Pythium* spp. associated with cavity spot are correctly identified so that appropriate control measures can be recommended.

We report a survey of *P.* spp. from cavity spot and other symptoms on carrots to determine whether *P. sulcatum* is associated with this disease in Eastern Australia. If *P. sulcatum* is associated with these symptoms, then the control measures devised in WA are potentially portable to other carrot growing regions in Australia.

[1] Advanced Bio Diagnostics, 21 Riga Crescent, Willetton Western Australia 6155
[2] Agriculture Victoria, IHD, Private Bag 15, South East Mail Centre, Vic 3176
[3] SARDI, GPO Box 397, Adelaide, SA 5001
[4] Agriculture Victoria, PO Box 905, Mildura, Vic 3502
[5] Serve-Ag Research, PO Box 690, Devonport, Tas 7310
[6] DPI, 80 Meiers Road, Indooroopilly, Qld 4068
[7] Elizabeth Macarthur Agricultural Institute, PMB 8, Camden, NSW 2570

Materials and methods

Fungal isolates

Pythium isolates from cavity spot, discoloured and forked roots and soil from carrot fields, were collected by collaborators in Queensland (QLD), New South Wales (NSW), Victoria (VIC), South Australia (SA) and Tasmania (TAS), and forwarded to WA for identification. A list of these isolates is given in Table 1.

Morphological identification and growth rate

Hyphal tip cultures were maintained on cornmeal agar (CMA) at 20^0C. Sporulation on colonised carrot seedlings, description of colony morphology and measurement of growth rate were made as described by Davison and McKay (1998). Isolates were grown on potato carrot agar (PCA) (Plaats-Niterink, 1981) for oospore production, and were identified using the keys of Plaats-Niterink (1981) and Dick (1990).

Molecular identification

A sub-set of isolates was provided to Advanced Bio Diagnostics for comparison with standard isolates of *P. sulcatum* and *P. violae* by RE-PCR, using the method of Wang and White (1997). DNA was extracted and amplified using the ITS1 and ITS4 primer pair. Amplification products were digested separately with Taq1, EcoR1 and HaeIII, and electorphoresed on 5 per cent acrylamide gel.

In vitro pathogenicity testing

Mature carrots cv Ivor, were hand harvested from a commercial property, washed in tap water, and stored overnight in a cold room at 4^0C. The next day they were surface sterilised with 2 per cent bleach for 2 mins, rinsed with distilled water, and placed in humid incubation chambers (Vivoda *et al.*, 1991).

Carrots were inoculated with a 5 mm disc of a 3 day old CMA culture of the test fungus placed upside down on the upper surface of the carrot. In each test there were 23 test fungi, one positive control (*P. sulcatum* isolate WAC9659) and a disc of CMA as a negative control. There were five inoculation positions on each carrot, five carrots in each incubation chamber and five replicate inoculations of each test fungus, for each of two incubation temperatures. Each inoculation chamber was placed in a large polythene bag and incubated at 15^0 or 22^0C for 6 days.

At harvest the maximum length and maximum width of each lesion was measured with a ruler. Where no lesion had formed this was recorded, and the lesion size recorded as the diameter of the agar disc (5 x 5 mm).

After measurement a small piece of tissue from the margin of each lesion was removed, surface sterilized for 5 sec. in 70 per cent ethanol, washed in sterile distilled water, dried on sterile paper, and plated onto CMA. Plates were incubated at 20^oC and examined after 3 and 7 days.

Statistical analysis

Lesion lengths and widths were compared by ANOVA using temperature*treatment for treatment, and temperature.chamber/treatment for block.

Table 1. *Pythium* spp. isolated from carrots in Australia

Accession number	WAC number*	Identity	Molecular identity	Host	Symptom	State	Post code	Isolated by**	Date	Lesions on carrot
P259	WAC9650	*P. sulcatum*	*P. sulcatum*	Carrot	Hairy root	NSW	2330	LT	1987	Yes
P672	WAC9653	*P. sulcatum*	*P. sulcatum*	Carrot	Forking	NSW	2250	LT	1992	Yes
P674		*P. torulosum*		Carrot	Forking	NSW	2250	LT	1992	No
P692		*P. irregulare*		Carrot	Forking	NSW	2871	LT	1991	At 22 C
P703		*P. ultimum*		Carrot	Forking	NSW	2330	LT	1987	No
P704		*P.* HS-group		Carrot	Forking	NSW	2330	LT	1987	No
P705	WAC9654	*P.* F-group	*P. sulcatum*	Carrot	Forking	NSW	2330	LT	1987	Yes
P706		*P.* F-group	*P. sulcatum*	Carrot	Forking	NSW	2330	LT	1987	Yes
P707		*P. ultimum*		Carrot	Forking	NSW	2330	LT	1987	No
P708		*P.* HS-group		Carrot	Forking	NSW	2330	LT	1987	No
P710		*P. ultimum*		Carrot	Forking	NSW	2330	LT	1987	No
P715	WAC9655	*P. sulcatum*	*P. sulcatum*	Carrot	Forking	NSW	2250	LT	1987	Yes
P718	WAC9656	*P. sulcatum*	*P. sulcatum*	Carrot	Forking	NSW	2330	LT	1987	Yes
P719		*P.* F-group	*P. sulcatum*	Carrot	Forking	NSW	2330	LT	1987	Yes
P773		*P.* F-group	Type 3	Carrot	Forking	NSW	2250	LT	1992	No
P774	WAC9657	*P. sulcatum*	*P. sulcatum*	Carrot	Forking	NSW	2250	LT	1992	Yes
99/353C	WAC9666	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	SA	5341	ED	1998	Yes
99/353E		*P. sulcatum*		Carrot	Cavity spot	SA	5341	ED	1998	Yes
99/353F		*P. sulcatum*		Carrot	Cavity spot	SA	5341	ED	1998	Yes
99/354A		*P. ultimum*		Carrot	Cavity spot	SA	5341	ED	1998	No
99/354B	WAC9667	*P. violae*	*P. violae*	Carrot	Cavity spot	SA	5341	ED	1998	Yes
99/354D		*P. violae*		Carrot	Cavity spot	SA	5341	ED	1998	Yes
99/354E	WAC9668	*P. violae*	*P. violae*	Carrot	Cavity spot	SA	5341	ED	1998	Yes
99/354G		*P. violae*		Carrot	Cavity spot	SA	5341	ED	1998	Yes
99/356A		*P. irregulare*		Carrot	Cavity spot	VIC	3549	ED	1998	No
99/357A		*P.* HS-group		Carrot	Cavity spot	VIC	3549	ED	1998	No
99/358A		*P. irregulare*		Carrot	Cavity spot	VIC	3549	ED	1998	Yes
99/358B		*P. irregulare*		Carrot	Cavity spot	VIC	3549	ED	1998	Yes
99/553B	WAC9669	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	QLD	4309	ED	1998	Yes

Table 1 continued

Accession number	WAC number*	Identity	Molecular identity	Host	Symptom	State	Post code	Isolated by**	Date	Lesions on carrot
99/553C		*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	QLD	4309	ED	1998	Yes
99/553D		*P. sulcatum*		Carrot	Cavity spot	QLD	4309	ED	1998	Yes
99/554A		*P. sulcatum*		Carrot	Cavity spot	QLD	4309	ED	1998	Yes
99/3839	WAC9670	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	SA	5355	RC	1998	Yes
99/3840	WAC9671	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	SA	5341	RC	1999	Yes
99/3841A	WAC9672	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	SA	5341	RC	1999	Yes
99/3841B		*P. sulcatum*		Carrot	Cavity spot	SA	5341	RC	1999	Yes
99/4739	WAC9673	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	TAS	7310	HP	1999	Yes
99/4740	WAC9674	*P. sulcatum*	*P. sulcatum*	Carrot	Black rot	TAS	7310	HP	1999	Yes
99/4741		*P.* ?		Carrot	Cavity spot	TAS	7310	HP	1999	At 22 C
99/4742		*P.* aff. *irregulare*		Carrot	Indentations	TAS	7307	HP	1999	At 15 C
99/4743		*P.* HS-group		Carrot	Indentations	TAS	7307	HP	1999	No
99/4744		*P.* HS-group		Carrot	Cavity spot	TAS	7307	HP	1999	No
99/4745A	WAC9675	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	TAS	7260	HP	1999	Yes
99/4745B	WAC9676	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	TAS	7260	HP	1999	Yes
99/4745C	WAC9677	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	TAS	7260	HP	1999	Yes
99/4745D	WAC9678	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	TAS	7260	HP	1999	Yes
99/4746	WAC9679	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	TAS	7310	HP	1999	Yes
99/4747	WAC9680	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	TAS	7310	HP	1999	Yes
99/5165A		*P. irregulare*		Carrot	Cavity spot	WA	6255	ED	1999	At 22 C
99/5165B		*P.* F-group		Carrot	Cavity spot	WA	6255	ED	1999	No
99/5168A		*P. sulcatum*		Carrot	Cavity spot	WA	6230	ED	1999	
99/5168B	WAC9682	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	WA	6230	ED	1999	Yes
99/5169D	WAC9683	*P. sulcatum*	*P. sulcatum*	Parsnip	Spot	WA	6230	ED	1999	Yes
99/5169E		*P. polymastum*		Parsnip	Spot	WA	6230	ED	1999	Yes
99/5361A	WAC9681	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	WA	6041	ED	1999	Yes
99/5361B		*P. sulcatum*		Carrot	Cavity spot	WA	6041	ED	1999	Yes
99/5768	WAC9684	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3939	RB	1999	Yes
99/5769	WAC9711	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3939	RB	1999	Yes
99/5770		*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3939	RB	1999	Yes
99/5771		*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3939	RB	1999	Yes

Table 1 continued

Accession number	WAC number*	Identity	Molecular identity	Host	Symptom	State	Post code	Isolated by**	Date	Lesions on carrot
99/5772		*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3939	RB	1999	Yes
99/5773		*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3939	RB	1999	Yes
99/5774	WAC9685	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3939	RB	1999	Yes
99/5775		*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3912	RB	1999	Yes
99/5776	WAC9686	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3912	RB	1999	Yes
99/5777		*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3912	RB	1999	Yes
99/5778	WAC9687	*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3795	RB	1999	Yes
99/5779		*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3795	RB	1999	Yes
99/5780		*P. sulcatum*	*P. sulcatum*	Carrot	Cavity spot	VIC	3795	RB	1999	Yes
99/6518A		*P.* F-group	Type 3	Carrot	Cavity spot	QLD	4362	RO'B	1999	At 22 C
99/6518B		*P.* F-group	Type 3	Carrot	Cavity spot	QLD	4362	RO'B	1999	Yes
99/6518C	WAC9688	*P. sulcatum*		Carrot	Cavity spot	QLD	4362	RO'B	1999	Yes
99/6519A		*P.* 'D'		Carrot	Lesion	QLD	4309	RO'B	1999	No
99/6520A		*P.* HS-group		Carrot	Brown roots	QLD	4310	RO'B	1999	Yes
99/6520B	WAC9689	*P.* F-group	*P. sulcatum*	Carrot	Brown roots	QLD	4310	RO'B	1999	Yes
99/6520C		*P.* F-group	Type 3	Carrot	Brown roots	QLD	4310	RO'B	1999	At 22 C
99/6520D		*P.* HS-group		Carrot	Brown roots	QLD	4310	RO'B	1999	Yes
99/6520E		*P.* 'D'		Carrot	Brown roots	QLD	4310	RO'B	1999	Yes
99/6520F		*P.* 'D'		Carrot	Brown roots	QLD	4310	RO'B	1999	At 22 C
99/6520G		*P.* 'D'		Carrot	Brown roots	QLD	4310	RO'B	1999	No
99/6521		*P.* HS-group		Carrot	Brown roots	QLD	4309	RO'B	1999	At 22 C
99/6565A		*Phytophthora megasperma*		Carrot	Black lesion	WA	6256	ED	1999	Yes
99/6586A	WAC9690	*P. sulcatum*	*P. sulcatum*	Carrot	Fork	NSW	2680	LT	1999	Yes
99/6586B	WAC9709	*P. sulcatum*	*P. sulcatum*	Carrot	Fork	NSW	2680	LT	1999	Yes
99/6586C	WAC9710	*P. sulcatum*	*P. sulcatum*	Carrot	Fork	NSW	2680	LT	1999	Yes
99/6587A	WAC9691	*P. sulcatum*	*P. sulcatum*	Carrot	Hairy root	NSW	2570	LT	1999	Yes
99/6587B		*P. sulcatum*		Carrot	Hairy root	NSW	2570	LT	1999	Yes
99/6587C		*P. sulcatum*		Carrot	Hairy root	NSW	2570	LT	1999	Yes
99/6587D		*P. acanthophoron*		Carrot	Fork and hairy root	NSW	2570	LT	1999	No
99/6588A		*P. sulcatum*		Carrot	Hairy root	NSW	2680	LT	1999	Yes

Table 1 continued

Accession number	WAC number*	Identity	Molecular identity	Host	Symptom	State	Post code	Isolated by**	Date	Lesions on carrot
99/6588B		*P.* HS-group		Carrot	Hairy root	NSW	2680	LT	1999	Yes
99/6678A	WAC9692	*P.* F-group	*P. sulcatum*	Carrot	Cavity spot	QLD	4344	RO'B	1999	Yes
99/6678B		*P. sulcatum*		Carrot	Discoloured root	QLD	4344	RO'B	1999	Yes
99/6678C		*P. sulcatum*		Carrot	Discoloured root	QLD	4344	RO'B	1999	Yes
99/6678D	WAC9712	*P. sulcatum*		Carrot	Discoloured root	QLD	4344	RO'B	1999	Yes
99/6678E		*P. sulcatum*		Carrot	Cavity spot	QLD	4344	RO'B	1999	Yes
99/6678F		*P. sulcatum*		Carrot	Cavity spot	QLD	4344	RO'B	1999	Yes
99/6678G		*P. sulcatum*		Carrot	Cavity spot	QLD	4344	RO'B	1999	Yes
99/6679A		*P.* HS-group		Carrot	Discoloured root tip	QLD	4344	RO'B	1999	At 22 C
99/6679B		*P.* HS-group		Carrot	Discoloured root tip	QLD	4344	RO'B	1999	Yes
99/6679C	WAC9693	*P.* F-group	*P. sulcatum*	Carrot	Discoloured root tip	QLD	4344	RO'B	1999	Yes
99/6679D		*P. sulcatum*		Carrot	Discoloured root tip	QLD	4344	RO'B	1999	Yes
99/6679E		*P.* F-group		Carrot	Discoloured root tip	QLD	4344	RO'B	1999	Yes
99/7270A		*P. sulcatum*		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/7270B	WAC9694	*P. sulcatum*		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/7270C		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	At 22 C
99/7270D		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/7271A		*P.* HS-group		Carrot	Discoloured root	QLD	4310	RO'B	1999	At 22 C
99/7271B		*P. sulcatum*		Carrot	Discoloured root	QLD	4310	RO'B	1999	Yes
99/7271C		*P. sulcatum*		Carrot	Discoloured root	QLD	4310	RO'B	1999	Yes
99/7271D		*P.* HS-group		Carrot	Discoloured root	QLD	4310	RO'B	1999	At 22 C
99/7734A		*P. violae*	*P. violae*	Carrot	Cavity spot	VIC	3549	S-AH	1999	Yes
99/7734B		*P. violae*	*P. violae*	Carrot	Cavity spot	VIC	3549	S-AH	1999	Yes
99/7734C		*P. violae*		Carrot	Cavity spot	VIC	3549	S-AH	1999	Yes
99/7734D	WAC9695	*P. violae*		Carrot	Cavity spot	VIC	3549	S-AH	1999	Yes
99/7734E		*P. ultimum*		Carrot	Cavity spot	VIC	3549	S-AH	1999	Yes
99/7734F	WAC9696	*P. sulcatum*		Carrot	Cavity spot	VIC	3590	S-AH	1999	Yes
99/7734G		*P. sulcatum*		Carrot	Cavity spot	VIC	3590	S-AH	1999	Yes
99/7734H	WAC9697	*P. sulcatum*		Carrot	Cavity spot	VIC	3590	S-AH	1999	Yes
99/7734I		*P. sulcatum*		Carrot	Cavity spot	VIC	3590	S-AH	1999	Yes
99/7734J		*P. sulcatum*		Carrot	Cavity spot	VIC	3590	S-AH	1999	Yes

Table 1 continued

Accession number	WAC number*	Identity	Molecular identity	Host	Symptom	State	Post code	Isolated by**	Date	Lesions on carrot
99/7734K	WAC9698	*P. sulcatum*		Carrot	Cavity spot	VIC	3585	S-AH	1999	Yes
99/7734L		*P. sulcatum*		Carrot	Cavity spot	VIC	3585	S-AH	1999	Yes
99/7734M		*P.* HS-group		Carrot	Cavity spot	VIC	3585	S-AH	1999	No
99/7734N		*P.* HS-group		Carrot	Cavity spot	VIC	3585	S-AH	1999	No
99/7734O		*P. ultimum*		Carrot	Cavity spot	VIC	3494	S-AH	1999	No
99/8178A		*P.* HS-group		Carrot	Brown roots	NSW	2250	LT	1999	No
99/8178B		*P.* F-group		Carrot	Brown roots	NSW	2250	LT	1999	Yes
99/8178C		*P.* F-group		Carrot	Brown roots	NSW	2250	LT	1999	Yes
99/8178D		*P.* F-group	Type 3	Carrot	Brown roots	NSW	2250	LT	1999	No
99/8178E		*P.* F-group		Carrot	Brown roots	NSW	2250	LT	1999	Yes
99/8178F		*P.* F-group		Carrot	Brown roots	NSW	2250	LT	1999	Yes
99/8178G		*P. ultimum*		Carrot	Brown roots	NSW	2250	LT	1999	No
99/8332A		*P.* F-group		Carrot	Cavity spot	QLD	4310	RO'B	1999	Yes
99/8332B	WAC9699	*P.* F-group	*P. sulcatum*	Carrot	Cavity spot	QLD	4310	RO'B	1999	Yes
99/8332C		*P.* F-group	*P. sulcatum*	Carrot	Cavity spot	QLD	4310	RO'B	1999	Yes
99/8332D		*P.* F-group		Carrot	Cavity spot	QLD	4310	RO'B	1999	Yes
99/8333A		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8333B	WAC9700	*P.* F-group	*P. sulcatum*	Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8333C		*P.* F-group	*P. sulcatum*	Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334A		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334B		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334C		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334D		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334E		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334F		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334G		*P.* HS-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334H		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334I		*P.* HS-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334J		*P.* HS-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8334K		*P.* HS-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8335A		*P.* F-group		Carrot	Cavity spot	QLD	4310	RO'B	1999	Yes

Table 1 continued

Accession number	WAC number*	Identity	Molecular identity	Host	Symptom	State	Post code	Isolated by**	Date	Lesions on carrot
99/8335B		*P.* F-group		Carrot	Cavity spot	QLD	4310	RO'B	1999	Yes
99/8336A	WAC9701	*P. sulcatum*		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8336B		*P. sulcatum*		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8336C		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
99/8336D		*P.* F-group		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
00/47A		*P.* HS-group		Carrot	Fork	NSW	2570	LT	1999	No
00/47B		*P. irregulare*		Carrot	Fork	NSW	2570	LT	1999	At 22 C
00/47C		*P.* HS-group		Carrot	Fork	NSW	2570	LT	1999	No
00/47D		*P. irregulare*		Carrot	Hairy root	NSW	2570	LT	1999	At 22 C
00/47E		*P.* HS-group		Carrot	Hairy root	NSW	2570	LT	1999	No
00/104	WAC9651	*P. violae*		Carrot	Cavity spot	VIC	3549	SAH	1999	Yes
00/105B		*P. ultimum*		Carrot	Cavity spot	VIC	3494	SAH	1999	No
00/106A		*P. sulcatum*		Carrot	Cavity spot	VIC	3590	SAH	1999	Yes
00/106B	WAC9652	*P. sulcatum*		Carrot	Cavity spot	VIC	3590	SAH	1999	Yes
00/106C		*P. sulcatum*		Carrot	Cavity spot	VIC	3590	SAH	1999	Yes
00/247C	WAC9702	*P.* F-group		Carrot	Cavity spot	QLD	4311	RO'B	1999	Yes
00/247D		*P.* F-group		Carrot	Cavity spot	QLD	4311	RO'B	1999	Yes
00/247E		*P.* F-group		Carrot	Cavity spot	QLD	4311	RO'B	1999	Yes
00/250A		*P.* aff *coloratum*		Soil		TAS	7310	HP	1999	At 22 C
00/250B		*P.* aff *coloratum*		Soil		TAS	7310	HP	1999	At 22 C
00/250C		*P.* aff *coloratum*		Soil		TAS	7310	HP	1999	No
00/250D		*P.* aff *coloratum*		Soil		TAS	7310	HP	1999	No
00/250E		*P.* aff *coloratum*		Soil		TAS	7310	HP	1999	No
00/250F		*P.* aff *coloratum*		Soil		TAS	7310	HP	1999	No
00/251A		*P.* aff *coloratum*		Carrot	Ring rot	TAS	7316	HP	1999	No
00/559		*P. irregulare*		Carrot	Seedling	SA	5120	RC	1999	No
00/560A		*P. irregulare*		Carrot	Stem	SA	5290	RC	1999	No
00/560B		*P. irregulare*		Carrot	Stem	SA	5290	RC	1999	No
00/1304		*Phytophthora* Group VI		Carrot	Cavity spot	QLD	4309	RO'B	1999	Yes
00/1305	WAC9703	*P.* F-group		Carrot	Cavity spot	QLD	4310	RO'B	1999	Yes

Table 1 continued

Accession number	WAC number*	Identity	Molecular identity	Host	Symptom	State	Post code	Isolated by**	Date	Lesions on carrot
00/1306		*P.* F-group		Carrot	Cavity spot	QLD	4310	RO'B	1999	Yes
00/1307	WAC9704	*P.* F-group		Carrot	Cavity spot	QLD	4310	RO'B	1999	Yes
00/1308		*P.* F-group		Carrot	Cavity spot	QLD	4310	RO'B	1999	Yes
00/1565A	WAC9705	*P. violae*		Carrot	Cavity spot	VIC	3549	SAH	1999	Yes
00/1565B		*P. ultimum*		Carrot	Cavity spot	VIC	3549	SAH	1999	No
00/1565C		*P. ultimum*		Carrot	Cavity spot	VIC	3549	SAH	1999	No
00/1567A		*P. irregulare*		Carrot	Cavity spot	VIC	3494	SAH	1999	At 22 C
00/1567B		*P. irregulare*		Carrot	Cavity spot	VIC	3494	SAH	1999	Yes
00/1567C		*P. irregulare*		Carrot	Cavity spot	VIC	3494	SAH	1999	At 22 C
00/1568A	WAC9706	*P.* HS-group		Carrot	Cavity spot	VIC	3549	SAH	1999	Yes
00/1568B		*P. irregulare*		Carrot	Cavity spot	VIC	3549	SAH	1999	No
00/1568C	WAC9707	*P. violae*		Carrot	Cavity spot	VIC	3549	SAH	1999	Yes
00/1569A		*P. ultimum*		Carrot	Cavity spot	VIC	3549	SAH	1999	At 22 C
00/1569B		*P. ultimum*		Carrot	Cavity spot	VIC	3549	SAH	1999	No
00/1570A		*P.* F-group		Water		VIC	3494	SAH	1999	No
00/2572A	WAC9708	*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/2572B		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/2572C		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/2572D		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/2572E		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/2572F		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/3084A		*P. sulcatum*		Carrot	Cavity spot	VIC	3745	RB	2000	Yes
00/3084B		*P. sulcatum*		Carrot	Cavity spot	VIC	3745	RB	2000	Yes
00/3084C		*P. sulcatum*		Carrot	Cavity spot	VIC	3745	RB	2000	Yes
00/3084D		*P. sulcatum*		Carrot	Cavity spot	VIC	3745	RB	2000	Yes
00/3084E		*P. sulcatum*		Carrot	Cavity spot	VIC	3745	RB	2000	Yes
00/3084F		*P. sulcatum*		Carrot	Cavity spot	VIC	3745	RB	2000	Yes
00/3084G		*P. sulcatum*		Carrot	Cavity spot	VIC	3745	RB	2000	Yes
00/3084H		*P.* F-group		Carrot	Cavity spot	VIC	3745	RB	2000	Yes
00/3084I		*P. sulcatum*		Carrot	Cavity spot	VIC	3745	RB	2000	Yes
00/3084J		*P. sulcatum*		Carrot	Cavity spot	VIC	3745	RB	2000	Yes

Table 1 continued

Accession number	WAC number*	Identity	Molecular identity	Host	Symptom	State	Post code	Isolated by**	Date	Lesions on carrot
00/3085A		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/3085B		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/3085C		*P.* F-group		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/3085D		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/3085E		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/3085F		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/3085G		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/3085H		*P. sulcatum*		Carrot	Cavity spot	VIC	3939	RB	2000	Yes
00/3934		*P. sulcatum*		Carrot	Cavity spot	TAS	7310	HP	2000	Yes
00/6062		*P. ultimum*		Carrot	Ring rot	TAS	7310	HP	2000	

*WAC: Western Australian Collection number

** RB: Robyn Brett, Agriculture Victoria; RC: Robin Cole, South Australian Research and Development Institute; ED: Elaine Davison, Deprtment of Agriculture Western Australia; S-AH: Sally-Ann Henderson, Agriculture Victoria; HP: Hoong Pung, Serve-Ag Research Tasmania; RO'B: Rob O'Brien, Queensland Department of Primary Industry; LT: Len Tesoriero, New South Wales Agriculture.

Results

Fungal isolates

Isolates from carrot symptoms and/or carrot soil were received from all states (Table 1, Table 2). Of the *Pythium* isolates, 67 per cent were from cavity spot lesions, 13 per cent were from discoloured roots and 10 per cent were from forked roots; 3 per cent were from soil or water. The remainder was from a range of symptoms such as hairy roots, indentations and ring rot (Table 1).

Table 2. Number of *Pythium* isolates examined.

State	Total number of isolates	Number of *Pythium* isolates
QLD	80	68*
NSW	37	37
VIC	116	73
SA	16	15
TAS	26	20
Total	275	213

* includes one *Phytophthora* isolate.

Morphological identification and growth rate

Pythium isolates were identified on the basis of their sexual and asexual organs. The most common species was *P. sulcatum*, accounting for 42.7 per cent of all isolates. Other isolates were *P. irregulare* (6.7 per cent), *P. ultimum* (5.6 per cent), *P. violae* (5.2 per cent), *P.* aff. *coloratum* (3.3 per cent), *P.* 'D' (1.9 per cent) and *P. torulosum* and *P. acanthophoron* each at 0.5 per cent.

Not all isolates formed oogonia, and so could not be identified to species. Some of these isolates (21.6 per cent) formed filamentous sporangia, and were identified as *Pythium* F-group (Dick, 1990). Some isolates (11.7 per cent) did not form oogonia or sporangia, and were identified as *Pythium* HS-group (Dick, 1990). One isolate was identified as *Phytophthora* Group VI. The complete list of identifications is given in Table 1.

There were differences in the radial growth of the species at 20^0C (Table 3). *P. sulcatum* and *P. violae* were slower growing than the other named species. *Pythium* F-group could be subdivided into two groups: those with a daily growth of less than 10 mm, and those which grew faster. Daily radial growth of *Pythium* HS-group ranged from 5.8–22.1 mm day^{-1}, and did not fall into discrete groups.

Table 3. Daily radial growth of the species at 20^{0}C on CMA.

Identity	Number of isolates	Radial growth at 20^{0}C (mm day^{-1})
P. sulcatum	91	7.9
P. irregulare	13	19.6
P. ultimum	12	18.0
P. violae	11	9.7
P. aff *coloratum*	7	16.4
P. 'D"	4	15.3
Pythium F-group, slow	39	7.2
Pythium F-group, fast	7	15.0
Pythium HS-group	24	14.2

Molecular identification

A sub-set of isolates was compared by RE-PCR with standard *P. sulcatum* and *P. violae* isolates (Table 4). The *P. sulcatum* isolates were confirmed as *P. sulcatum*, and the *P. violae* isolates were confirmed as *P. violae*. Slow growing *Pythium* F-group isolates were identified as *P. sulcatum* while fast growing isolates were designated as Type 3 (Table 4).

Table 4. Comparison of morphological and molecular identifications of *Pythium* isolates from carrots.

Morphological identification	Molecular identification	Number of isolates	Radial growth at 20^{0}C (mm day^{-1})
P. sulcatum	*P. sulcatum*	21	9.5
P. violae	*P. violae*	3	10.0
Pythium F-group, slow	*P. sulcatum*	10	7.5
Pythium F-group, fast	Type 3	5	15.1

In vitro pathogenicity testing

All isolates were used in *in vitro* pathogenicity tests on carrots at 15^0 and 22^{0}C. Isolates were classes as pathogenic if the mean lesion length and width was significantly greater ($P<0.05$) than the negative control. All *P. sulcatum, P. violae* and slow growing *Pythium* F-group were pathogenic at both temperatures (Table 5). Other frequently isolated species (*P. irregulare, P. ultimum, P.* aff. *coloratum, P.* 'D', fast growing *Pythium* F-group and *Pythium* HS-group) gave variable results (Table 5).

Table 5. ***In vitro*** **pathogenicity of isolates on carrots at 15^0 and 22^{0}C.** Complete results are given in Table 1

Morphological identity	Number of isolates	Pathogenicity at: 15^0 & 22^{0}C	15^{0}C only	22^{0}C only	Non-pathogenic
P. sulcatum	91	91			
P. irregulare	14	3	1	5	5
P. ultimum	12	1		1	10
P. violae	11	11			
P. aff. *coloratum*	7			2	5
P. 'D'	4	1		1	2
P. torulosum	1				1
P. acanthophoron	1				1
P. F-group, slow	39	39			
P. F-group, fast	7	1		3	3
P. HS-group	24	9		4	11
Phytophthora Group VI	1	1			

Discussion

The two *Pythium* spp. that are the most important cause of cavity spot world wide are *P. violae* and *P. sulcatum* (White, 1986; Nagai *et al.,* 1986; Montfort and Rouxel, 1988; Vivoda *et al.*, 1991; Breton and Rouxel, 1993; Benard and Punja, 1995). Both species are important in North America and Europe (Kalu *et al.,* 1976; White, 1988; White *et al.,* 1993; Benard and Punja, 1995), while only *P. sulcatum* has been reported from Japan (Nagai *et al.,* 1986; Kaygeyama *et al.*, 1996). Although both species occur in Western Australia (Dewan and Sivasithamparam, 1989; El-Tarabily *et al.,* 1996), only *P. sulcatum* has been isolated from carrots (El-Tarabily *et al.,* 1996; Davison and McKay, 1998).

In this survey of *Pythium* spp. associated with cavity spot and symptoms on carrots, there are three taxa *P. sulcatum, P. violae* and slow growing *P.* F-group that are consistently pathogenic in *in vitro* pathogenicity tests (Table 5). Molecular characterisation of slow growing *P.* F-group shows that this is *P. sulcatum* (Table 4).

P. sulcatum was the most common species, present in all states, and isolated from most carrot growing regions (Table 6, Figure 1). In Queensland it was most commonly in the asexual form, and therefore identified morphologically as *P.* F-group, while in other states most isolates produced oogonia in culture, and could therefore be identified as *P. sulcatum* (Table 6). *P. violae* was isolated from only two areas both adjacent to the Murray River (Table 6, Figure 1). Carrot production is a relatively new industry in this wheat farming area, and wheat is a host of *P. violae* (Dewan and Sivasithamparam, 1989; Schrandt *et al.*, 1994). This is the first record of *P. violae* on carrots in Australia.

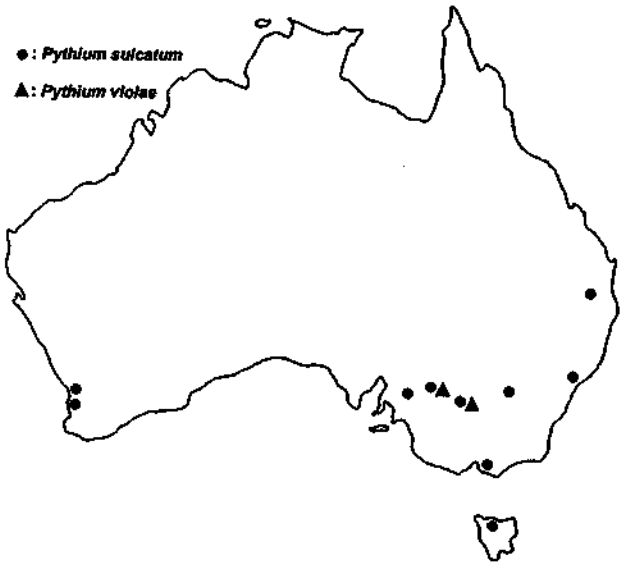

Figure 1. The distribution of *Pythium sulcatum* and *P. violae* in carrot growing regions of Australia.

Table 6. Location, by postcode, of *P. sulcatum*, *P. violae* and slow growing *P.* F-group isolates from carrots.

State	Postcode	Number of *P.* isolates	Number of isolates of: *P. sulcatum*	*P. violae*	*P.* F-group, slow
NSW	2250	12	3		4
	2330	10	2		3
	2570	9	3		
	2680	5	4		
	2871	1			
VIC	3494	6			
	3549	18		7	
	3585	4	2		
	3590	8	8		
	3745	10	9		1
	3795	3	3		
	3912	3	3		
	3939	21	20		1
QLD	4303	29	8		13
	4310	21	2		11
	4311	3			3
	4344	12	7		3
	4362	3	1		
SA	5120	1			
	5290	2			
	5341	11	6	4	
	5355	1	1		
TAS	7260	4	4		
	7307	3			
	7310	12	5		
	7316	1			
Total		213	91	11	39

1.2 Molecular characterisation of *Pythium sulcatum* isolates from carrots

Summary Fifty-six *Pythium sulcatum* isolates from all carrot growing regions were compared by RAPD analysis, with type and isotype cultures. *P. violae* was used as the outgroup. A neighbour joining tree showed that the Australian isolates formed three clusters (1, 3 and 4), while the type and isotype clustered separately (cluster 2). These four clusters differed in growth rate, oogonial diameter, oospore diameter and aplerotic index. Australian cluster 4 has a smaller proportion of monoclinous antheridia than clusters 1 and 3.

There was geographical separation of some of the clusters. Isolates from Tasmania were only in cluster 1, while isolates from Queensland were only in cluster 4. Isolates from Western Australia were in clusters 1 and 3, those from southern Victoria were in clusters 3 and 4. Isolates from New South Wales and the River Murray were in cluster 1, 3 and 4.

This diversity of isolates suggests that *P. sulcatum* is a cosmopolitan species that may occur on native Australian Apiaceae.

Introduction

Cavity spot disease is one of the most important diseases of carrots worldwide. It is caused by *Pythium* spp., notably *P. violae* and *P. sulcatum* (White, 1986; Nagai *et al.*, 1986; Montfort and Rouxel, 1988; Vivoda *et al.*, 1991; Breton and Rouxel, 1993; Benard and Punja, 1995). In Australia *P. sulcatum* is the most widespread cause of this disease, occurring in all of the commercial carrot growing areas (Section 1.1). Isolation records (Plaats-Niterink, 1981) and field experiments (McKay and Davison, 2000; 99MD18) have shown that the host range of *P. sulcatum* is restricted to Apiaceae. Although *P. violae* has a much wider host range than *P. sulcatum* (Plaats-Niterink, 1981) it has only been isolated from carrots growing on farms along the River Murray in Victoria and South Australia (Section 1.1).

Commercial carrot production in Australia occurs on a small number of sites where there are appropriate soils and adequate rainfall or water for irrigation. The restricted host range of *P. sulcatum,* together with the small number of areas where carrots are grown, might indicate that *P. sulcatum* has been introduced into these area on contaminated seed or equipment. Alternatively, it might be a cosmopolitan pathogen of Apiaceae that was present on sites before carrots were grown.

If *P. sulcatum* is an introduced pathogen then it may be possible to exclude it from new carrot growing areas. If however, it occurs on native Australian Apiaceae and/or umbelliferous weeds, then exclusion is not an option for disease control.

A study of the genetic uniformity of *P. sulcatum* may indicate whether it is an introduced or cosmopolitan species.

Materials and Methods

Fungal isolates

The isolates used were *P. sulcatum* together with slow growing *P.* F-group, that were pathogenic to carrots. The selection was based on isolates that had been collected in different seasons from carrot growing regions throughout Australia (Table 7). Hyphal tip cultures were maintained on corn meal agar (CMA).

DNA extraction

Pythium isolates WAC9653, WAC9684, WAC9685, WAC9686, WAC9687, WAC9690 and WAC9691 were grown in V8 liquid medium (200 ml of V8 vegetable juice [Campbell's Soups Australia], 800 ml of water, pH adjusted to 5.5 with NaOH, autoclaved) for 5 to 10 days at 26°C. The hyphal mat was removed, washed several times in sterile distilled water, pressed dry between filter papers and freeze dried prior to storage at – 20°C. Before extraction of DNA, the hyphal mat was dropped into liquid nitrogen and then ground to a fine powder in a small flat bottomed plastic test-tube, using the blunt end of a spatula. The hyphal powder was placed in a 1.7 ml Effendorf tube and mixed with 400 μL of extraction buffer (200 mM Tris HCl pH 8.5, 250 mM NaCl, 25 mM EDTA Na_2, 0.5per cent SDS (Raeder and Broda, 1985)).

To extract the DNA, the mixture was incubated at 65°C for 2 hours and then centrifuged at 1400 rpm for 15 min. To purify and concentrate the DNA (Vogelstein and Gillespie, 1979), 200 μL of the supernatant was added to 600 μL of sodium iodide (1 g mL^{-1}) containing 5 μL of glass milk (http://bionet.hgmp.mrc.ac.uk/hypermail/methods/methods.199209/1493.html). The mixture was vortexed to ensure suspension of the glass milk, incubated at room temperature for 15 min with occasional shaking and then centrifuged for 10 sec. The supernatant was discarded and the pellet resuspended in 800 μl of washing liquid (100 nM NaCl, 10 mM Tris pH7.5, 1 mM EDTA, 50per cent EtOH), centrifuged for 10 sec and the supernatant discarded. This was repeated. The pellet was resuspended in 600 μL of cold EtOH (100per cent), precipitated by centrifuging for 60 sec, the EtOH was discarded and the tubes drained for about 30 min. The pellet was then resuspended in 40 μl of TE, incubated at 50°C for 10 min before centrifuging for 2 min. 25μL of the supernatant was removed and an aliquot diluted 1/25 in sterile water prior to storage at – 20°C.

All the remaining test isolates were grown on CMA until the colony had reached the edge of the petri dish. A piece of colonised agar (20 x 10 mm) was removed, placed in a 1.7 mL Effendorf tube, crushed using a hand-held pellet pestle grinder. Two or three drops from 400 μL of extraction buffer were added and the mixture ground using a motorised pellet pestle grinder. The remaining extraction buffer was added and the mixture vortexed prior to freezing (– 20°C). To extract the DNA, the mixture was thawed, incubated at 65°C for 2 hours and then treated as described above.

Table 7. *Pythium* isolates used in the molecular analysis.

WAC number*	Accession no.	Identity	Molecular cluster	Isolated from	Location	Isolated by	Date
WAC9644	CBS178.86	*P. violae*	Out group	Carrot	Netherlands	Blok	1986
WAC9646	CBS603.73	*P. sulcatum* type	2	Carrot	Wisconsin, USA	Pratt & Mitchell	1973
WAC9647	CBS604.73	*P. sulcatum* isotype	2	Soil	Florida, USA	Pratt & Mitchell	1973
WAC9648	IMI366698	*P. sulcatum*	1	Carrot	WA 6035#	KET**	1994
WAC8470	WAC8470	*P. sulcatum*	1	Carrot	WA 6041	AG	1994
WAC8760	WAC8760	*P. sulcatum*	1	Carrot	WA 6041	RMF	1995
WAC9650	P259	*P. sulcatum*	1	Carrot	NSW 2330	LT	1987
WAC9653	P672	*P. sulcatum*	3	Carrot	NSW 2250	LT	1992
WAC9654	P705	*P. sulcatum*	3	Carrot	NSW 2330	LT	1987
WAC9655	P715	*P. sulcatum*	3	Carrot	NSW 2250	LT	1987
WAC9656	P718	*P. sulcatum*	1	Carrot	NSW 2330	LT	1987
WAC9657	P774	*P. sulcatum*	3	Carrot	NSW 2250	LT	1992
WAC9658	2588A	*P. sulcatum*	3	Carrot	WA 6041	ED	1996
WAC9659	2616AD	*P. sulcatum*	1	Carrot	WA 6171	ED	1996
WAC9660	2698A	*P. sulcatum*	1	Carrot	WA 6171	ED	1996
WAC9661	2720D	*P. sulcatum*	1	Carrot	WA 6041	ED	1996
WAC9662	2721D	*P. sulcatum*	1	Carrot	WA 6041	ED	1996
WAC9663	2772A	*P. sulcatum*	1	Carrot	WA 6035	ED	1996
WAC9664	2916A	*P. sulcatum*	3	Carrot	WA 6035	ED	1996
WAC9665	2918A	*P. sulcatum*	1	Carrot	WA 6041	ED	1996
WAC9666	99/353C	*P. sulcatum*	4	Carrot	SA 5341	ED	1998
WAC9669	99/553B	*P sulcatum*	4	Carrot	QLD 4309	ED	1998
WAC9670	99/3839	*P. sulcatum*	4	Carrot	SA 5355	RC	1998
WAC9671	99/3840	*P. sulcatum*	1	Carrot	SA 5341	RC	1999
WAC9672	99/3841A	*P. sulcatum*	1	Carrot	SA 5341	RC	1999
WAC9673	99/4739	*P. sulcatum*	1	Carrot	TAS 7310	HP	1999
WAC9674	99/4740	*P. sulcatum*	1	Carrot	TAS 7310	HP	1999
WAC9675	99/4745A	*P. sulcatum*	1	Carrot	TAS 7260	HP	1999
WAC9676	99/4745B	*P. sulcatum*	1	Carrot	TAS 7260	HP	1999
WAC9677	99/4745C	*P. sulcatum*	1	Carrot	TAS 7260	HP	1999
WAC9678	99/4745D	*P. sulcatum*	1	Carrot	TAS 7260	HP	1999
WAC9679	99/4746	*P. sulcatum*	1	Carrot	TAS 7310	HP	1999

Table 7 continued

WAC number*	Accession no.	Identity	Molecular cluster	Isolated from	Location	Isolated by	Date
WAC9680	99/4747	*P. sulcatum*	1	Carrot	TAS 7310	HP	1999
WAC9681	99/5361A	*P. sulcatum*	3	Carrot	WA 6041	ED	1999
WAC9682	99/5168B	*P sulcatum*	3	Carrot	WA 6230	ED	1999
WAC9683	99/5169D	*P. sulcatum*	3	Parsnip	WA 6230	ED	1999
WAC9684	99/5768	*P. sulcatum*	3	Carrot	VIC 3939	RB	1999
WAC9685	99/5774	*P. sulcatum*	3	Carrot	VIC 3939	RB	1999
WAC9686	99/5776	*P. sulcatum*	3	Carrot	VIC 3912	RB	1999
WAC9687	99/5778	*P. sulcatum*	3	Carrot	VIC 3795	RB	1999
WAC9688	99/6518C	*P. sulcatum*	4	Carrot	QLD 4362	RO'B	1999
WAC9689	99/6520B	*P. sulcatum*	3	Carrot	QLD 4310	RO'B	1999
WAC9690	99/6586A	*P. sulcatum*	4	Carrot	NSW 2680	LT	1999
WAC9691	99/6587A	*P. sulcatum*	3	Carrot	NSW 2570	LT	1999
WAC9692	99/6678A	*P. sulcatum*	4	Carrot	QLD 4344	RO'B	1999
WAC9693	99/6679C	*P. sulcatum*	4	Carrot	QLD 4344	RO'B	1999
WAC9694	99/7270B	*P. sulcatum*	3	Carrot	QLD 4309	RO'B	1999
WAC9696	99/7734F	*P. sulcatum*	1	Carrot	VIC 3590	SAH	1999
WAC9697	99/7734H	*P. sulcatum*	1	Carrot	VIC 3590	SAH	1999
WAC9698	99/7734K	*P. sulcatum*	3	Carrot	VIC 3585	SAH	1999
WAC9699	99/8332B	*P. sulcatum*	4	Carrot	QLD 4310	RO'B	1999
WAC9700	99/8333B	*P. sulcatum*	4	Carrot	QLD 4309	RO'B	1999
WAC9701	99/8336A	*P. sulcatum*	4	Carrot	QLD 4309	RO'B	1999
WAC9652	00/106B	*P. sulcatum*	3	Carrot	VIC 3590	SAH	1999
WAC9702	00/247C	*P.* F-group	4	Carrot	QLD 4311	RO'B	1999
WAC9703	00/1305	*P.* F-group	4	Carrot	QLD 5636	RO'B	1999
WAC9704	00/1307	*P.* F-group	4	Carrot	QLD 5636	RO'B	1999
WAC9706	00/1568A	*P.* HS-group	4	Carrot	VIC 3549	SAH	1999
WAC9708	00/2572A	*P. sulcatum*	4	Carrot	VIC 3939	RB	2000

*WAC: Western Australian Collection number

** AG: Angie Galati, Department of Agriculture Western Australia; ED: Elaine Davison, Department of Agriculture Western Australia; HP: Hoong Pung, Serve-Ag Research Tasmania; KET: Kahlid El-Tarabily, Murdoch University, Western Australia; LT: Len Tesoriero, New south Wales Agriculture; RB: Robyn Brett, Agriculture Victoria; RC: Robin Coles, South Australian Research and Development Institute; RMF: Rob Floyd, Department of Agriculture Western Australia; RO'B: Rob O'Brien, Queensland Department of Primary Industry; SAH: Sally-Ann Henderson, Agriculture Victoria.

State and post code: NSW: New South Wales; QLD: Queensland; SA: South Australia; TAS: Tasmania; VIC: Victoria; WA: Western Australia

PCR

DNA from all isolates was amplified using five OPC random primers (OPC-1, -4, -6, -18 and -19; Operon Kit C, Operon Technologies, Alameda, USA) individually. The PCR reaction (9 μL) consisted of 5.727 μL of water, 2 μL of polymerisation buffer (x 5), 0.8 μL $MgCl_2$ (25 mM), 0.4 μL primer (25 p mole μL^{-1}), 0.073 μL T*th* plus DNA polymerase (1 unit). 1μL of the diluted DNA was added as template to the 9 μL PCR reaction mixture. PCR reactions were conducted in 0.3 mL tubes (Omnistrip, 8 tubes per strip; ABgene, Surrey, UK) using a Hybraid Om*ni*Gene thermal cycler with heated lids and block temperature control. Blocks were preheated to 50°C for 8 min before placing the reaction tubes in the thermal cycler. The PCR cycles consisted of 25 sec of denaturing at 94°C, 1 min of annealing at 32°C, and 1 min of extension at 72°C repeated 10 times followed by 25 cycles of the same program except the annealing was increased to 45°C for 30 sec. The PCR products were separated by electrophoresis through 6 per cent acrylamide non-denaturing gel in TBE buffer at 40 volt x hours cm^{-1}, stained with ethidium bromide (1 μg ml^{-1}) and photographed under UV light.

To characterise variation in banding patterns between the isolates, a matrix was constructed recording the presence or absence (1 or 0) of bands. The analysis was performed on a total of 134 bands (OPC-1, 32 bands; -4, 11; -6, 40; -18, 19; -19, 32). Relatedness of the isolates was determined by combining the results from the five PCRs using RAPDistance programme version 1.04 with algorithm #1 (Dice, 1945; Nei and Li, 1979) to generate a Neighbour Joining tree (Armstrong *et al.*, 1994).

Morphological characters

The characters that had been used for identification (Section 1.1) were compared for isolates in different molecular clusters.

Statistical analysis

Analysis of variance (GENSTAT version 5, 1993) was used to compare growth rates and morphological characteristics of the molecular clusters. As the sample sizes were unequal, pairwise *t*-tests were performed to make contrasts between clusters if there was overall significance in the ANOVA table.

Results

RAPD analysis

RAPD analysis showed that the *P. sulcatum* isolates clustered into four groups (Table 7, Figure 2). The North American isolates (type and isotype) clustered together (cluster 2). There was some geographical clustering of the Australian isolates (Table 8). Queensland isolates were in cluster 4, Tasmanian isolates were in cluster 1, isolates from Western Australia were in clusters 1 and 3, those from southern Victoria were in clusters 3 and 4, while isolates from New South Wales and along the Murray River were in clusters 1, 3 and 4.

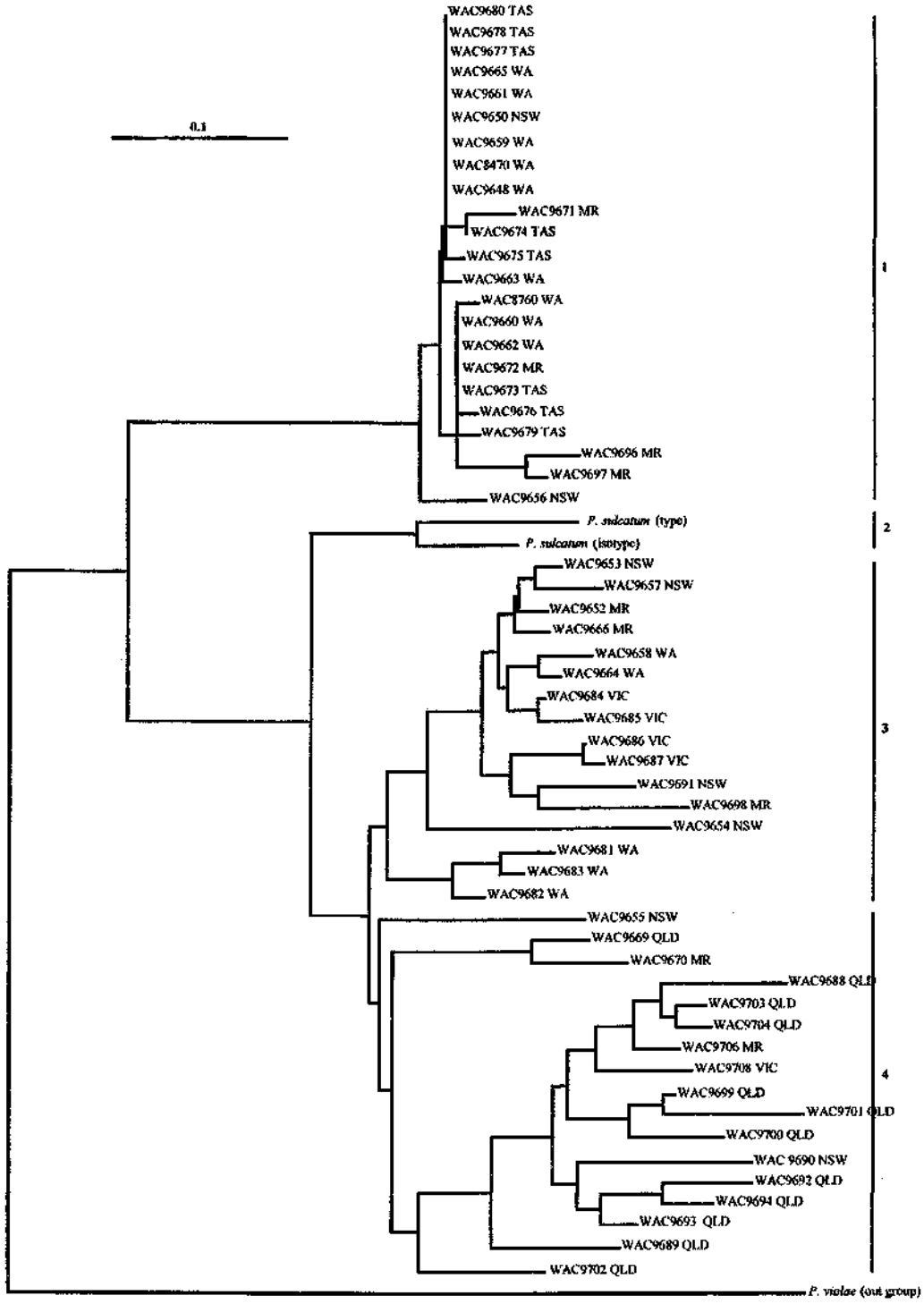

Figure 2. Neighbour Joining tree of *Pythium sulcatum* isolates.

Table 8. Geographical clustering of *P. sulcatum* isolates from Australia

Molecular cluster	NSW	QLD	Murray River	Southern VIC	TAS	WA
1	2	0	4	0	8	9
3	4	0	3	4	0	5
4	2	13	2	1	0	0
Sample size	8	13	9	5	8	14

Comparison of morphological characters

Observations of morphological characters and growth rate on CMA at 20^0C were compared for isolates in the different clusters (Table 9). As the type and isotype isolates did not form oogonia in culture, comparisons could either not be made, or were only made using data from the type description (Pratt and Mitchell, 1973). Not every Australian isolate formed oogonia in culture so comparisons between molecular clusters were not always made with the maximum sample size.

Table 9 shows that isolates from cluster 3 grew significantly faster than isolates from the other clusters. Clusters 1, 3 and 4 showed no difference in the proportion of terminal oogonia, but there were differences in the diameter of oogonia, oospores and aplerotic index. Clusters 1 and 4 had larger oogonia and oospores than clusters 2 and 3, and cluster 1 had a higher aplerotic index (Dick, 1990) than the other clusters. There was no difference in the proportion of oogonia with single antheridia, but there was a higher proportion of monoclinous antheridia in clusters 1 and 3 than in cluster 4.

Table 9. Comparison of mophometric data and growth rate at 20^{0}C for *Pythium sulcatum* isolates from carrot growing regions of Australia. Within columns values with the same superscript are not significantly different (*P>0.05*).

Molecular cluster	Maximum sample size	Growth rate (mm^{-day})	Oogonial diameter (µm)	Oospore diameter (µm)	Maximum wall thickness (µm)	Aplerotic index	Terminal oogonia (%)	Single antheridium (%)	Monoclinous antheridia (%)
1	23	5.8^a	20.0^a	19.4^a	2.0	0.91^a	90.7	65.2	75.8^a
2	2	5.3^a	16$^{b\#}$	14$^{b\#}$	1.5$^{\#}$	0.67^b			
3	16	8.4^c	18.2^b	16.5^b	1.7	0.76^b	95.7	63.7	77.7^a
4	17	7.1^{ab}	21.4^a	19.4^a	1.9	0.79^b	93.1	57.5	60.5^b
DF		53	37	34	34	34	40	40	40
Significance		***	***	***	n.s.	***	n.s.	n.s.	***
	Actual sample size								
1		22	18	18	18	18	21	21	21
2		2	1	1	1	1			
3		16	15	12	12	12	15	15	15
4		17	7	6	6	6	7	7	7

*** = *P*<0.001; ** = *P*<0.01; * = *P*<0.05; n.s. = *P*>0.05

values from type description

Discussion

Native or introduced?

Pythium is a large, cosmopolitan genus whose members occupy a wide range of diverse habitats. Some species, such as *P. irregulare* have a wide host range (Barr *et al.*, 1997; Plaats-Niterink, 1981), while others such as *P. sulcatum* only infect closely related plants. One of the aims of this study was to determine the genetic uniformity of *P. sulcatum* in Australia. If it is genetically uniform it would indicate that it is likely to be a recently introduced pathogen of carrots. If, however, it is genetically diverse, then it might be either a cosmopolitan pathogen of Apiaceae that occurs naturally on Australian members of this family, or it may have been introduced many times into the different carrot growing regions of Australia.

Our results show that *P. sulcatum* in Australia is not genetically uniform. There is some geographical clustering such as the isolates from Queensland that all cluster together (cluster 4, Table 8, Figure 2), but there are also isolates from different regions, such as Tasmania and Western Australia, that show identical RAPD profiles (cluster 1, Figure 2). This is much greater similarity than that found in *P. ultimum* where genetically distinct isolates occurred in the same soil sample, and infected the same tissue (Francis and St. Clair, 1997).

Thus it can be argued that there is evidence that *P. sulcatum* may be both a pathogen of native Apiaceae, but also have been moved around Australia with the carrot industry.

If *P. sulcatum* occurs on Australian Apiaceae it should be possible to find it on plants in native vegetation. It also indicates that cavity spot is likely to develop on carrot crops on sites that have been recently cleared.

How useful are mophometrics?

The classification of *Pythium* is based on the shape of sporangia, position and ornamentation of oogonia, and number and arrangement of antheridia. The size of the reproductive cells is not as widely used for *Pythium* as for other genera, because there has been considerable debate about its taxonomic value. Hendrix and Campbell (1974) questioned the value of such measurements because they found that the diameter of oogonia and oospores of several species changed after sub-culturing. Shazad *et al.* (1992) however, used oospore and oogonial diameters, together with aplerotic index, ooplast index and wall index, in canonical variate analysis to separate 80 isolates of 40 species. Ali-Shtayeh (1985) has shown that diameters of both oogonia and oospores of 155 isolates, representing 42 species, varied significantly between species, compared to variation within species, and considered that this justified their taxonomic value. However both Shazad *et al.* (1992) and Ali-Shtayeh (1985) were working with a small number of isolates of a large number of species. Our data show that the different molecular clusters of *P. sulcatum* differ significantly in oogonial and oospore diameter (Table 9), and support the view that these are of limited taxonomic value.

Other characters that are used to separate species include ornamentation of the oogonia, whether the oospores are plerotic or aplerotic, and number and position of the antheridia. In a study of *P. irregulare*, Biesbrock and Hendrix (1967) showed that the proportion of oogonia with projections depended on whether they were formed on

the surface of agar, or within the medium. They also showed that the proportion of plerotic and aplerotic varied between isolates, as did the proportion of diclinous antheridia. Dick (1990) has accommodated this within-species variation in his key to the genus, by introducing the concept of modal arrangement and number. Our observations of *P. sulcatum* show that all molecular clusters have a similar proportion of terminal oogonia, and of oogonia with a single antheridium (Table 9). The modal arrangement of all clusters is monoclinous, even though there are significant differences in the proportion of monoclinous antheridia in different molecular clusters (Table 9).

Pratt and Mitchell (1973) describe *P. sulcatum* as aplerotic. Shahzad *et al.* (1992) point out that this corresponds to an oospore that occupies no more than 60–65 per cent of the oogonial volume. Our calculations from the original data (Pratt and Mitchell, 1973) give a value of 67 per cent (Table 9). Australian isolates, however, have a much higher aplerotic index (76-91 per cent, Table 9) so would be more accurately described as plerotic. Barr *et al.* (1997) have shown that the aplerotic index of *P. irregulare* is also variable, giving values of 66.2-98.5 per cent for 124 isolates.

Implications for the Australian carrot industry

Our study of *P. sulcatum* shows that there is considerable variation in *P. sulcatum* from around Australia. All isolates however, were pathogenic in *in vitro* pathogenicity tests (Table 5) so in spite of showing both morphological and genetic variation, all must be regarded as important pathogens.

Section 2. Victorian and South Australian carrot surveys

R. Brett[8], R. Cole[9] and E. M. Davison

2.1 Survey of carrot crops in Victoria and South Australia for cavity spot disease.

Summary Three carrot crops in Victoria and eight crops in South Australia were surveyed in 1999 and 2000 for quality and incidence of cavity spot, using a protocol developed in Western Australia. The mean weight of carrots, from 1 m row plots, varied from 0.8 to 5.9 kg. Irrespective of the presence of cavity spot, the proportion of export quality carrots varied from 37 to 91 per cent. The main defects were misshapen carrots (10.5 per cent) and forked or stumped carrots (7.7 per cent). The incidence of cavity spot varied from 0 to 79 per cent, and was more common in the crops from Victoria than those from South Australia.

Introduction

The *Pythium* disease cavity spot, is a major constraint on the production of high quality carrots. In a survey carried out in Western Australia in 1990/91 (Galati and McKay, 1996), cavity spot was most severe on intensively cropped sites with poor rotation. Although cavity spot occurs on carrots in other Australian states (Section 1.1), there have been no surveys of its importance. Small surveys of mature carrot crops in the Southern Victoria and carrot growing regions of South Australia were undertaken as part of HRDC project VG98011. The results of these surveys are reported here.

Methods

The survey was conducted on mature, commercial carrot crops. Six sample plots were sampled from a random walk across a field. Each plot consisted of a 1 m length of row, from the centre or one of the centre rows of a raised bed. The carrots were hand-harvested, washed and graded in the following manner: carrots were sorted into cavity spot ratings of 0, 1, 2, 3 and 4 or more spots, then each rating was sorted into the following categories: export marketable (>150 mm long, 25-50 mm crown diameter), short marketable (120-150 mm long, 25-50 mm crown diameter), undersize (<120 mm long or <25 mm crown diameter), oversize (>50 mm crown diameter), forked or stumped, misshapen, split. The number and weight of carrots in each grade was recorded for each cavity spot rating.

[8] Institute for Horticultural Development, Agriculture Victoria, Private Bag 15, South East Mail Centre, Vic. 3180
[9] South Australian Research and Development Institute, GPO Box 367, Adelaide, SA 5001

Results

Three crops were sampled in Victoria and eight crops were sampled in South Australia. The crops were sampled in spring, summer and autumn (Table 10, Table 11).

There were considerable differences amongst the crops, with the weight ranging from 0.8 to 5.9 kg (Table 10) and the number of carrots ranging from 8 to 48.5 (Table 11) per m length of row. The proportion of export marketable yield, irrespective of cavity spot, ranged from 37 to 91 per cent by weight (Table 10). The main defects were misshapen and forked or stumped carrots (Table 10, Table 11).

Cavity spot occurred in some of the surveyed crops. The incidence in the Victorian crops varied from 27 to 80 per cent by weight (Table 10). In the South Australian crops it was either absent or less than 2 per cent (Table 10, Table 11).

Discussion

This small survey has shown that there is huge variation in the incidence of cavity spot in different carrot growing regions. The survey is too small, however, to draw firm conclusions.

Table 10. The weight of mature carrots, and proportion of different grades irrespective of cavity spot, and proportion of carrot with cavity spot irrespective of grade.

State	Postcode	Date	Variety	Total weight (kg)	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)
Vic	3795	10.1.00	Viking	4.9	37.2	0.1	5.9	0.0	9.5	32.7	14.6	79.5
Vic	3939	25.3.99		2.5	49.1	7.2	14.5	0.0	12.6	12.0	4.6	73.7
Vic	3939	10.1.00		5.9	40.2	2.8	1.3	1.3	25.7	23.6	5.1	27.4
SA	5120	2.6.00	Ricardo	2.1	53.2	28.1	8.7	8.3	4.5	0.0	0.0	0.0
SA	5120	2.6.00	Ricardo	2.1	88.8	6.1	3.3	0.0	0.8	1.0	0.0	0.6
SA	5157	14.6.00		5.9	90.9	0.7	1.4	5.6	0.6	0.9	0.0	0.0
SA	5341	27.10.99	Crusader	1.4	71.1	1.8	1.3	0.0	9.9	3.6	12.3	1.5
SA	5355	14.3.00	Stefano	0.8	88.1	0.0	2.2	0.0	0.0	10.1	0.0	0.0
SA	5355	2.5.00	Carrisma	1.2	79.8	0.0	1.6	6.2	0.0	12.4	0.0	1.6
SA	5357	14.6.00	Carrisma	1.2	41.2	9.9	1.2	7.0	21.4	19.2	0.0	0.0
SA	5357	12.11.99	Stefano	1.7	91.4	5.1	3.5	0.0	0.0	0.0	0.0	0.0

Table 11. The number of mature carrots, and proportion of different grades irrespective of cavity spot, and proportion of carrot with cavity spot irrespective of grade.

State	Postcode	Date	Variety	Total number	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)
Vic	3795	10.1.00	Viking	42.2	31.6	0.4	12.7	0.0	10.9	32.2	12.3	73.7
Vic	3939	25.3.99		30.3	36.2	7.9	35.1	0.0	9.3	8.2	3.2	71.9
Vic	3939	10.1.00		48.5	37.7	4.5	4.1	0.8	24.1	24.6	4.2	24.9
SA	5120	2.6.00	Ricardo	29.2	54.8	26.2	12.3	6.2	0.5	0.0	0.0	0.0
SA	5120	2.6.00	Ricardo	23.5	76.0	8.8	12.7	0.0	1.0	1.5	0.0	1.0
SA	5157	14.6.00		33.2	87.3	1.9	6.2	3.1	0.9	0.5	0.0	0.0
SA	5341	27.10.99	Crusader	15.5	65.5	2.7	4.7	0.0	8.3	5.7	13.1	1.0
SA	5355	14.3.00	Stefano	8.0	83.6	0.0	9.5	0.0	0.0	6.9	0.0	0.0
SA	5355	2.5.00	Carrisma	12.8	85.1	0.0	4.7	1.8	0.0	8.4	0.0	1.2
SA	5357	14.6.00	Carrisma	10.5	32.8	14.8	4.9	8.0	23.8	15.7	0.0	0.0
SA	5357	12.11.99	Stefano	25.3	75.0	9.0	16.0	0.0	0.0	0.0	0.0	0.0

Section 3. Cultural methods for controlling *Pythium* diseases of carrots: host range, rotation, solarisation.

E. M. Davison, P. A. Murphy and A. G. McKay

3.1 Host range of *Pythium sulcatum* (99md18)

Summary. Seedlings of several potential hosts of *Pythium sulcatum*, the cause of cavity spot in Western Australia, were grown in infested soil at the Medina Research Station. *Pythium* spp. were isolated from seedling roots when the plants were 6 weeks old. *P. sulcatum* was only isolated from carrot seedlings and one bean plant. Grasses (barley, maize, oats, rye and wheat) and cucurbits (cucumber and musk melon) were not infected.

Introduction

Pythium sulcatum is the main cause of cavity spot of carrots in Western Australia (Davison and McKay, 1998). *P. sulcatum* was first described from carrots in the USA. (Pratt and Mitchell, 1973), and published records show that it has been isolated from carrots in many countries (Watanabe *et al.*, 1986; White, 1986; Guerin *et al.*, 1994; Benard and Punja, 1995) and from parsley (Plaats-Niterink, 1981).

Most *Pythium* spp. have wide host ranges so that rotation is not a satisfactory way to reduce the incidence of disease. *P. sulcatum* however, has a restricted host range because a previous experiment (McKay and Davison, 2000) showed that only members of the Apiaceae (carrot, celery, parsley, parsnip, *Trachymene coerulea*) were infected by *P. sulcatum* when grown in infested soil at the Medina Research Station. Unrelated plants (beetroot, broccoli, capsicum (*Capsicum annuum*), lettuce, onion and tomato) were not infected, although *P. sulcatum* was isolated from 1 per cent of sampled spinach roots.

Results of this experiment at the Medina Research Station differ from those reported from Canada. Kalu *et al.* (1976) carried out a similar experiment in a glasshouse, using Canadian isolates of *P. sulcatum*. They sowed a range of vegetables into steam-sterilized muck soil that had been infested with pure cultures of *P. sulcatum*. They found root dieback on roots of young plants, and were able to re-isolate *P. sulcatum* from both symptomatic and symptomless roots. The most severe root dieback was on members of the Apiaceae (carrot, celery, parsley and parsnip) and bean (*Phaseolus vulgaris*), but they also reported some infection of cucumber (*Cucumis sativus*), eggplant (*Solanum melongena* var. *fructescens*), lettuce (*Lactuca sativa*), musk melon (*Cucumis melo*), onion (*Allium cepa*) and pepper (*Capsicum frutescens*). They recorded that maize, tomato and cabbage were not infected.

Many carrot growers seed wheat or cereal rye to give wind protection to carrot seedlings or use oats in rotation with carrots. Although preliminary sampling

(Davison unpublished) has shown that these are not infected by *P. sulcatum*, it is important to confirm that these are non-hosts.

In this experiment we have firstly determined whether *P. sulcatum* is associated with roots of the cereals used by carrot growers, and secondly, whether vegetables, from plant families other than Apiaceae, are hosts of *P. sulcatum*. These vegetables include some of those reported as hosts by Kalu *et al.* (1976).

Materials and methods

Growth of seedlings

The experiment was established at the Medina Research Station, 30 km south of Perth. It was a randomised block design with four replicates. Each 3 m long experimental plot comprised two double rows of the test species, with two double rows of the susceptible carrot cultivar Ivor as buffers. The potential hosts used are shown in Table 12. The experiment was seeded on 15.1.01.

Table 12. Potential hosts of *P. sulcatum* used in the experiment.

Host	Common name	Variety
Daucus sativa	Carrot	Ivor
Cucumis melo	Musk melon	Hales Best
C. sativus	Cucumber	Burpless
Phaseolus vulgaris	Bean	Brown Beauty
Avena sativa	Oats	Mortlock
Hordeum vulgare	Barley	Mighty Mouse
Secale cereale	Rye	
Triticum aestivum	Wheat	Eradu
Zea mais	Maize	Honeysweet

Seedling harvest

The experiment was harvested on 27.2.01. Twenty seedlings were carefully removed from each plot, and stored overnight at 4^0C in polythene bags. The following day the tap roots were carefully washed with tap water, lateral roots removed, each tap root was cut into 1 cm long pieces and five pieces per seedling plated directly onto *Pythium*-selective agar (corn meal agar amended with 100 mg ampicillin, 1 ml nystatin, 0.5 ml rifampicin, 100 mg PCNB per L). In the case of the cereal seedlings, 1 cm long pieces from 5 crown roots were plated out onto selective agar. Plates were incubated for 2 days at room temperature, and a further 3 days at 15^0 C. *Pythium* spp. were identified on their colony and hyphal morphology; *P. sulcatum* isolates were identified from their slow growth, colony and hyphal morphology.

Statistical analysis

The incidence and severity of infection was compared by analysis of variance (GENSTAT version 5, 1993).

Results

Comparison of hosts

Pythium spp. were isolated from all hosts; oats had the highest incidence and severity (Table 13). *P. sulcatum* was isolated from only carrots and a single bean root piece (Table 13).

Table 13. Incidence (proportion of seedlings infected) and severity (proportion of root pieces infected) of *Pythium* infection of seedling roots.

Host	*Pythium* spp.		*P. sulcatum*	
	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
Carrot	15	4.3	14	4.0
Musk melon	40	4.0	0	0.0
Cucumber	30	7.3	0	0.0
Bean	39	9.5	1	0.3
Oats	56	23.5	0	0.0
Barley	36	8.8	0	0.0
Rye	45	14.5	0	0.0
Wheat	41	13.0	0	0.0
Maize	55	17.5	0	0.0
Significance	*	0.07	n.s.	n.s.
LSD (0.05)	25			

* = $P<0.05$, n.s. = $P>0.05$

Discussion

This experiment indicates that cereals used for wind protection in carrot crops are not hosts of *P. sulcatum*, even though they have a high incidence of infection by other species of *Pythium*. These results differ from those of Kalu *et al.* (1976) because there was no infection of cucumber, or musk melon. Although bean was infected, only root piece from one plant out of 80 sampled plants yielded *P. sulcatum*.

The host range of WA isolates of *P. sulcatum* as determined from field experiments, differs from that of Canadian isolates assessed in the glasshouse.

3.2 The use of rotation for controlling *Pythium* diseases of carrots (99md2)

Summary. *Pythium sulcatum* causes cavity spot of carrots in Western Australia. Control by rotation with a non-host, broccoli, was attempted on a badly infested site at the Medina Research Station. Carrots were planted after one, two or three broccoli crops. The incidence and severity of seedling infection by *P. sulcatum* was significantly reduced when carrots followed broccoli. At harvest this was associated with decreased forking and increased root length, resulting in an increase in export yield. There was a decrease in the incidence and severity of cavity spot in two of the three plantings where carrots followed broccoli, but these results were inconsistent. Oospores of *P. sulcatum* are able to survive for at least 21 months in the absence of a host.

Introduction

Pythium sulcatum, the cause of cavity spot in Western Australia (WA), infects closely related plants in the family Apiaceae, but not unrelated plants (Davison and McKay 2000, Section 3.1). One way to reduce the incidence of cavity spot, would be to use a non-host in rotation with carrots.

A site at Medina Research Station was infested with cavity spot affected carrots in 1996, and has been continuously cropped with the susceptible cultivar Ivor. Grid surveys of each crop have shown that the incidence of cavity spot has increased in successive crops. In the crop harvested in December 1998, 62 per cent of carrots had cavity spot.

The aim of this experiment was to determine whether the incidence and severity of cavity spot and other diseases caused by *P. sulcatum,* could be reduced by rotation with broccoli, a non-host.

Materials and Methods

Experimental site

A 0.24 ha site at Medina Research Station was infested with cavity spot affected carrots in 1996 and repeatedly cropped with the susceptible cultivar Ivor. A grid survey to assess uniformity of infestation was conducted in November 1996, June 1997, December 1997 and December 1998, just before each crop was harvested. A sample of 12 carrots, three from each double row per bed, was hand harvested at 10 m intervals along each bed, starting at 5 m from the western end of each bed. The carrots were hand washed and stored in a cold room at 1^0C. Each carrot was assessed for cavity spot, and the incidence calculated for each sample point.

A widespread *Sclerotinia* infestation on the site was treated with metham sodium at 500 L ha^{-1}, 10 days before the site was seeded with the first planting.

A severe weed infestation on the site at the end of the second planting was controlled with metham sodium at 500 L ha^{-1}, 6 weeks before the site was re-seeded.

Experimental design

This trial covered a sequence of four consecutive crops in which carrots (cv Ivor) were rotated with broccoli (cv Marathon for winter crops and cv Greenbelt for

summer crops) in different combinations (Table 14). The outside beds (beds 1 and 14) of the experimental site were treated as experiment buffers. The experimental plots comprised three beds, 1.5 m wide by 10 m long, the outside beds were treated as plot buffer beds. There was a 5 m uncultivated buffer between the plots. In order to minimise the risk of soil movement between adjacent plots, all machinery was washed or brushed down between treatments. Each treatment was replicated four times, and the treatments were blocked down the beds.

Table 14. Carrot/broccoli crops in successive plantings.

	Planting			
	1	2	3	4
Seeding date	13.04 99	30.09.99	16.03.00	1.09.00
Seedling harvest	10.05.99	10.11.99	9.05.00	31.10.00
Harvest	27.8.99	20.01.00	1.08.00	18.12.00
Treatment 1	Carrots	Carrots	Carrots	Carrots
Treatment 2	Broccoli	Carrots	Broccoli	Carrots
Treatment 3	Carrots	Broccoli	Carrots	Broccoli
Treatment 4	Broccoli	Broccoli	Carrots	Carrots
Treatment 5	Carrots	Broccoli	Broccoli	Carrots
Treatment 6	Broccoli	Broccoli	Broccoli	Carrots

Crop maintenance

Basal fertiliser (superphosphate at 1500 kg ha^{-1} and trace elements at 150 kg ha^{-1}) was incorporated into the soil 1 week before seeding. Nitrogen, potassium and magnesium were applied weekly through the irrigation, commencing 1 week after seeding. Total crop nitrogen ranged from 310 to 416 kg N ha^{-1}, applied as a mixture of potassium nitrate and sulphate of ammonia. Total crop potassium ranged from 300 to 310 kg K ha^{-1}, applied as potassium nitrate. Total crop magnesium ranged from 24 to 30 kg Mg ha^{-1}, applied as magnesium sulphate.

Dacthal® was used for weed control in both carrots and broccoli.

Raw carrot seed was sown with an Agricola air seeder to give four double rows per 1.5 m wide raised bed, with a target density of 60 plants $m^{2\ -1}$. Broccoli was seeded with an Agricola seeder to give two single rows per bed. The broccoli seedlings were thinned after 6 weeks to a final in-row spacing of 24 cm.

At the end of each rotation, carrots from the centre beds were hand harvested, the roots removed, and the tops returned to the experimental beds. Carrots from the buffer beds were hand harvested and removed completely from the site. Broccoli tops were mulched with a flail mower so that the leaves would breakdown rapidly. The stumps, with attached roots, were hand pulled and left on the soil for several days to dry out, before being removed from the plots.

Seedling harvests

A linear sample of 20 seedlings was taken from one of the middle two rows of carrots, and from one row of broccoli, for root isolations. The roots were washed thoroughly, and examined for lesions. Each tap root was cut into 1 cm pieces, and five pieces plated directly onto agar selective for *Pythium* (section 3.1). All plates were incubated at room temperature. *Pythium* growing from the roots were characterised on the basis of colony morphology, growth rate, sporangial morphology, and morphology of the oogonia, antheridia and oospores.

Final harvest

At harvest, four 1 m lengths of row from one of the middle rows were hand harvested, placed in onion bags, machine washed, and then stored in a cold room at 1^0C until they were assessed.

The quality of the bulk crop was assessed by number and by weight into the following categories: export marketable (>150 mm long, 25-50 mm crown diameter), short marketable (120-150 mm long, 25-50 mm crown diameter), undersized (<120 mm long, or <25 mm crown diameter), oversize (>50 mm crown diameter), forked, misshapen, split. These categories were used for each cavity spot rating of 0, 1, 2, 3 and 4 or more spots.

Isolations were made from a random sample of about 50 carrots. One spot from each carrot was plated onto agar selective for *Pythium*, and any isolates characterised as described above.

Statistical analysis

GENSTAT version 5 (1993) was used to analyse the results using transformed or untransformed data as appropriate.

The analysis of the incidence and severity of seedling infection was analysed using block as the block stratum, and crop as the treatment stratum. For the final harvest, the incidence and severity of cavity spot was analysed using block/treatment/sub-sample as the block stratum, and treatment as the treatment stratum.

Results

Incidence of cavity spot on the experimental site

Survey data showed that the incidence of cavity spot increased with time since site infestation (Table 15).

Table 15. Incidence of cavity spot in the beds used for the experimental plots.

Date	Incidence of cavity spot (%)				
	Bed 3	Bed 6	Bed 9	Bed 12	Mean
Nov. 1996	15.8	5.8	5.8	11.7	9.8
June 1997	17.5	10.8	16.7	23.3	17.1
Dec. 1997	23.3	25.8	41.7	41.7	32.9
Dec. 1998	74.2	60.8	62.5	74.2	67.9

Seedling harvests

Pythium spp. were isolated from some, but not all, of the carrot and broccoli seedlings (Table 16).

P. sulcatum was isolated more frequently from carrots than broccoli; it was only isolated from a single root segment from a single broccoli plant in plantings 2 and 4 (Table 16). Both incidence and severity of infection was significantly lower in carrots seedlings that followed a broccoli crop than those in the control treatment (Table 16).

Final harvest, planting 1

There was no difference in the total weight or number of carrots harvested from the different treatments (Table 17, Table 18). When cavity spot was ignored, there was no difference in the proportion of export marketable, short marketable, undersize, oversize, forked, misshapen or split carrots between treatments (Table 17, Table 18). There was no difference between treatments in the incidence of cavity spot, irrespective of carrot quality, or severity of cavity spot, assessed as number of spots per carrot (Table 17, Table 18).

Pythium was isolated from 89 per cent of 55 sampled cavity spots. All isolates were *P. sulcatum.*

Final harvest, planting 2

The total weight and number of carrots harvested from the different treatments were similar (Table 19, Table 20). When cavity spot was ignored, there was a significant increase in export yield, associated with a decrease in forking, in the crop that followed broccoli (treatment 2), compared with the crop following carrots (treatment 1). There was a decrease in the incidence of cavity spot, and there were fewer spots per carrot in treatment 2 compared with treatment 1 (Table 19, Table 20).

Pythium was isolated from 72 per cent of 60 sampled cavity spots. All isolates were *P. sulcatum.*

Final harvest, planting 3

The total weight and number of carrots harvested from the different treatments was similar (Table 21, Table 22). When cavity spot was ignored, there was a significant increase in export yield in the carrot crop that followed either one (treatment 3) or two (treatment 4) crops of broccoli. There was also a significant decrease in the proportion of short and forked carrots. There was a decrease in the incidence of cavity spot, and the number of spots per carrot, in treatment 4 compared with treatments 1 and 3 (Table 21, Table 22).

Pythium was isolated from 23 per cent of 35 sampled cavity spots. All isolates were *P. sulcatum.*

Table 16. Plantings 1, 2, 3 and 4. Infection of seedlings by *Pythium*. Incidence is the proportion of seedlings from which *P.* spp. were isolated, severity is the number of root pieces per seedling from which *P. sulcatum* was isolated. Analyses were performed on untransformed data.

Treatment	Crop	Incidence of *P.* spp. (%)	Incidence of *P. sulcatum* (%)	Severity of *P. sulcatum* (%)
Planting 1				
1	Carrots	27.5	25.0	10.0
2	Broccoli	3.7	0.0	0.0
3	Carrots	32.5	32.5	14.3
4	Broccoli	2.5	0.0	0.0
5	Carrots	20.0	20.0	7.0
6	Broccoli	10.0	0.0	0.0
Significance		n.s.	n.s.	n.s.
Planting 2				
1	Carrots	82.5	70.0	29.0
2	Carrots	47.5	8.7	1.8
3	Broccoli	27.5	1.2	0.3
4	Broccoli	65.0	0.0	0.0
5	Broccoli	51.3	0.0	0.0
6	Broccoli	58.8	0.0	0.0
Significance		***	***	***
LSD (P<0.05)		18.5	13.9	9.2
Planting 3				
1	Carrots	72.5	68.8	22.0
2	Broccoli	61.3	0.0	0.0
3	Carrots	36.3	33.8	8.5
4	Carrots	15.0	8.7	2.0
5	Broccoli	76.3	0.0	0.0
6	Broccoli	75.0	0.0	0.0
Significance		***	***	***
LSD (P<0.05)		18.4	11.7	5.4
Planting 4				
1	Carrots	62.5	60.0	15.8
2	Carrots	37.5	30.0	6.8
3	Broccoli	33.8	1.2	0.3
4	Carrots	32.5	18.8	4.8
5	Carrots	25.0	12.5	3.0
6	Carrots	23.8	7.5	1.5
Significance		*	***	***
LSD (P<0.05)		22.1	15.1	4.3

*** = P< 0.001, ** = P< 0.01, * = P< 0.05, n.s. = P>0.05

Table 17. Planting 1. Comparison of the weight of mature carrots (cv Ivor), proportion of different grades of carrot irrespective of cavity spot, and proportion of carrots with cavity spot irrespective of grade.

Treatment	Previous crop	Total weight (kg)	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)
1	Carrots	2.9	61.8	17.5	4.7	0.7	1.6	8.2	5.4	82.5
3	Carrots	2.9	61.8	19.6	5.0	1.7	1.3	7.5	3.1	79.4
5	Carrots	2.9	62.3	19.1	4.6	0.0	1.0	8.6	4.5	79.2
Significance		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*** = $P< 0.001$, ** = $P< 0.01$, * = $P< 0.05$, n.s. = $P>0.05$

Table 18. Planting 1. Comparison of the number of mature carrots (cv Ivor), proportion of different grades of carrot irrespective of cavity spot, and proportion of carrots with cavity spot irrespective of grade.

Treatment	Previous crop	Total number	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)	Cavity spots per carrot
1	Carrots	27.0	50.6	21.2	11.6	0.2	2.0	9.4	4.9	80.0	2.7
3	Carrots	27.7	51.2	25.7	10.8	0.8	1.7	7.1	2.8	80.0	2.5
5	Carrots	27.0	49.7	23.8	11.5	0.0	0.9	9.2	5.0	78.4	2.5
Significance		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*** = $P< 0.001$, ** = $P< 0.01$, * = $P< 0.05$, n.s. = $P>0.05$

Table 19. Planting 2. Comparison of the weight of mature carrots (cv Ivor), proportion of different grades of carrot irrespective of cavity spot, and proportion of carrots with cavity spot irrespective of grade.

Treatment	Previous crop	Total weight (kg)	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)
1	Carrots	2.7	56.6	4.8	0.5	4.0	9.2	20.0	4.8	92.0
2	Broccoli	3.3	69.1	4.5	2.4	2.0	2.9	15.6	3.6	68.1
Significance		n.s. (0.09)	*	n.s.	**	n.s.	n.s. (0.06)	*	n.s.	**

*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, n.s. = $P > 0.05$

Table 20. Planting 2. Comparison of the number of mature carrots (cv Ivor), proportion of different grades of carrot irrespective of cavity spot, and proportion of carrots with cavity spot irrespective of grade.

Treatment	Previous crop	Total number	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)	Cavity spots per carrot
1	Carrots	18.1	49.6	7.1	1.6	2.2	13.0	21.9	4.6	88.2	3.4
2	Broccoli	21.8	61.6	5.9	6.7	0.9	4.1	17.8	2.9	66.2	2.3
Significance		n.s.	*	n.s.	n.s. (0.08)	n.s.	*	n.s.	n.s.	**	*

*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, n.s. = $P > 0.05$

Table 21. Planting 3. Comparison of the weight of mature carrots (cv Ivor), proportion of different grades of carrot irrespective of cavity spot, and proportion of carrots with cavity spot irrespective of grade.

Treatment	Previous crops	Total weight (kg)	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)
1	C+C+C	3.1	62.7	20.3	4.5	0.6	9.2	2.1	0.6	70.0
3	C+C+B	3.2	79.6	8.9	2.9	1.3	2.7	3.9	1.5	65.9
4	C+B+B	3.4	85.7	6.5	1.3	1.2	1.8	2.8	0.7	36.6
Significance		n.s.	*	**	n.s.	n.s.	**	n.s.	n.s.	*
lsd			13.0	7.3			4.2			11.1

*** = $P< 0.001$, ** = $P< 0.01$, * = $P< 0.05$, n.s. = $P>0.05$

Table 22. Planting 3. Comparison of the number of mature carrots (cv Ivor), proportion of different grades of carrot irrespective of cavity spot, and proportion of carrots with cavity spot irrespective of grade.

Treatment	Previous crop	Total number	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)	Cavity spots per carrot
1	C+C+C	22.7	52.4	24.0	10.3	0.2	9.5	2.9	0.6	69.6	2.4
3	C+C+B	22.3	66.9	13.5	8.1	0.6	3.2	6.6	1.1	66.7	2.3
4	C+B+B	21.4	79.8	10.0	3.5	0.5	2.1	3.5	0.7	34.4	0.9
Significance		n.s.	**	**	n.s.	n.s.	*	n.s.	n.s.	*	*
lsd			11.7	5.6			4.4			27.4	1.1

*** = $P< 0.001$, ** = $P< 0.01$, * = $P< 0.05$, n.s. = $P>0.05$

Final harvest, planting 4

The yield, measured both by weight and number, was significantly less in the treatments where carrots followed carrots, compared with treatments where carrots followed broccoli (Table 23, Table 24). When cavity spot was ignored, there was a significant increase in the proportion of export yield in the carrot crop that followed broccoli crops (treatments 2, 5 and 6) (Table 23) compared with carrots that followed a carrot crop. There was a reduction in forking in carrots that followed at least two broccoli crops (Table 24).

There was no significant effect of broccoli crops on the incidence of cavity spot, or the number of spots per carrot (Table 23, Table 24).

P. sulcatum was isolated from 55 per cent of 40 sampled cavity spots. It was the only species of *Pythium* isolated.

Comparison of severity of cavity spot in the four harvests

When carrots were assessed, they were rated as having zero, one, two, three, or four or more spots. Comparisons of severity have been made between carrots with one to three spots and four or more spots (Table 25, Table 26). In the first planting there was no difference in the severity of cavity spot between treatments. In the second and third plantings there were significantly fewer carrots in the four or more class after one (planting 2, treatment 2) or two (planting 3, treatment 3) broccoli crops (Table 25, Table 26).

Table 23. Planting 4. Comparison of the weight of mature carrots (cv Ivor), proportion of different grades of carrot irrespective of cavity spot, and proportion of carrots with cavity spot irrespective of grade.

Treatment	Previous crops	Total weight (kg)	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)
1	C+C+C+C	1.8	60.9	2.9	0.7	2.9	16.7	7.8	8.1	86.1
2	C+B+C+B	2.7	64.1	8.9	1.4	0.5	8.0	8.5	8.2	97.3
4	C+B+B+C	2.2	62.1	1.3	0.3	0.0	19.4	7.3	9.6	91.9
5	C+C+B+B	3.0	75.3	5.8	1.4	0.0	4.1	9.8	3.5	89.3
6	C+B+B+B	3.2	76.2	4.1	0.9	0.0	8.5	7.4	2.9	73.5
Significance		***	*	*	n.s.	n.s.	0.05	n.s.	n.s.	n.s.
lsd		0.4	12.6	4.2						

*** = $P< 0.001$, ** = $P< 0.01$, * = $P< 0.05$, n.s. = $P>0.05$

Table 24. Planting 4. Comparison of the number of mature carrots (cv Ivor), proportion of different grades of carrot irrespective of cavity spot, and proportion of carrots with cavity spot irrespective of grade.

Treatment	Previous crop	Total number	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)	Cavity spots per carrot
1	C+C+C+C	11.1	55.3	4.9	2.4	1.9	20.5	8.4	6.6	82.9	3.4
2	C+B+C+B	21.3	56.1	11.8	4.4	0.3	11.1	9.1	7.3	95.0	4.3
4	C+B+B+C	12.1	57.2	2.4	1.5	0.0	22.3	9.4	7.2	89.2	3.7
5	C+C+B+B	21.8	67.2	8.3	4.3	0.0	5.7	11.8	2.7	86.9	3.5
6	C+B+B+B	22.0	71.0	6.5	2.9	0.0	9.7	7.4	2.6	71.6	2.9
Significance		***	0.06	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
lsd		3.9		5.6			11.1				

*** = $P< 0.001$, ** = $P< 0.01$, * = $P< 0.05$, n.s. = $P>0.05$

Table 25. Summary of the severity of cavity spot in all harvests, comparison by weight. Comparisons were made on arcsine transformed data, untransformed data are presented. Within columns, values with the same superscript are not significantly different ($P > 0.05$).

Treatment	Planting 1		Planting 2		Planting 3		Planting 4	
	Carrots with 1-3 spots (%)	Carrots with 4 or more spots (%)	Carrots with 1-3 spots (%)	Carrots with 4 or more spots (%)	Carrots with 1-3 spots (%)	Carrots with 4 or more spots (%)	Carrots with 1-3 spots (%)	Carrots with 4 or more spots (%)
1 (C+C+C+C+C)	41.5	39.6	30.3	60.7[a]	33.6	34.1[a]	17.7	68.4
2 (C+B+C+B+C)			30.3	35.4[b]			10.9	86.2
3 (C+C+B+C+B)	47.7	30.5			31.3	32.4[a]		
4 (C+B+B+C+C)					18.7	7.8[b]	18.4	74.4
5 (C+C+B+B+C)	43.0	34.1					22.2	67.3
6 (C+B+B+B+C)							19.8	50.2
Significance	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	n.s.

*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, n.s. = $P > 0.05$

Table 26. Summary of the severity of cavity spot in all harvests, comparison by number. Comparisons were made on arcsine transformed data, untransformed data are presented. Within columns, values with the same superscript are not significantly different ($P > 0.05$).

Treatment	Planting 1		Planting 2		Planting 3		Planting 4	
	Carrots with 1-3 spots (%)	Carrots with 4 or more spots (%)	Carrots with 1-3 spots (%)	Carrots with 4 or more spots (%)	Carrots with 1-3 spots (%)	Carrots with 4 or more spots (%)	Carrots with 1-3 spots (%)	Carrots with 4 or more spots (%)
1 (C+C+C+C+C)	41.8	36.7	32.3	54.9[a]	35.6	31.6[a]	19.3	62.8
2 (C+B+C+B+C)			31.9	31.9[b]			12.4	82.4
3 (C+C+B+C+B)	49.7	29.0			31.3	33.2[a]		
4 (C+B+B+C+C)					17.9	6.9[b]	22.2	67.8
5 (C+C+B+B+C)	43.4	32.7					24.1	62.4
6 (C+B+B+B+C)							20.6	47.6
Significance	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	n.s.

*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, n.s. = $P > 0.05$

Discussion

Rotation is a traditional method for controlling root diseases. To be successful, rotational crop(s) must be a non-host, and the period elapsing between susceptible crops must be longer that the survival time of the pathogen(s) of concern. Previous experiments on the host range of *P. sulcatum* have shown that broccoli seedlings are not infected when they are grown in infested field plots (McKay and Davison, 2000) and cabbage is not infected in a glasshouse experiment (Kalu *et al.,* 1976). We do not know how long *P. sulcatum* survives under field conditions.

The main diseases that are caused by *P. sulcatum* and *P. violae* on carrots are seedling infections which lead to forking and reduced plant density in the mature crop, and cavity spot (White 1986, Liddell *et al.*, 1989). The main concerns of the carrot industry in WA are cavity spot, and forking (Galati and McKay, 1996).

Our experiment has shown that there is a significant reduction in the incidence of seedling infection by *Pythium* spp., including *P. sulcatum,* when broccoli, not carrots are the previous crop (Table 16). Reduced seedling infection is associated with decreased forking and increased root length, resulting in an increase in export yield (Table 20, Table 22, Table 24).

In two of the three plantings where carrots followed either one or two broccoli crops, there is a significant decrease in incidence and severity of cavity spot (Table 20, Table 22, Table 26), however, this result is not consistent (Table 22, Table 24, Table 26). From the results of Planting 4, it is clear that oospores of *P. sulcatum* are able to survive for at least 21 months in the absence of a host.

Many brassicas contain significant quantities of glucosinolates that are hydrolysed to isothiocyanates when their tissues are disrupted. Isothiocyanates are biocidal to a wide range of organisms including many soil-borne pests and pathogens (Sawar *et al.,* 1998). Shetty *et al.* (2000) have shown that incorporation of broccoli residues reduces cauliflower wilt, caused by *Verticillium dahliae,* by reducing the viability of microsclerotia in soil. The broccoli used in our experiments contains 4-methylthiobutenyl- and 2-phenylethyl-isothiocyanate in its roots, although these are not present in the leaves (J. Matthiessen, pers. comm.). Mycelial growth of *P. sulcatum* is very sensitive to 2-phenylethyl-isothiocyanate (B. Smith, pers. comm.). If 2-phenylethyl-isothiocyanate is liberated into soil, either from growing broccoli roots or decaying tissue, we would expect inhibition of adjacent *P. sulcatum* hyphae. This may be the reason for the reduction in seedling infection, shown in Table 16. If there is a biofumigation effect on *P. sulcatum*, it is likely to be fungistatic, rather than fungitoxic, because broccoli crops do not consistently reduce the incidence and severity of cavity spot.

3.3 The potential for controlling cavity spot disease by soil solarisation (01pe1)

Summary. Solarisation is a cultural method for controlling soil-borne diseases where soil is heated by solar energy. The potential for solarisation to reduce cavity spot disease of carrots was assessed in a series of *in vitro* experiments that determined the thermal inactivation of *Pythium sulcatum* and *P. violae*. Isolates of *P. violae* failed to survive for 2 hr. at 35^{0}C while *P. sulcatum* survived for 2 hr. at 45^{0}C, and 6 hr. at 42.5^{0}C. In the field it is unlikely that temperatures achieved by solarisation will be high enough to reduce the inoculum potential of *P. sulcatum,* although these temperatures may be sufficient to reduce the inoculum of *P. violae*.

Introduction

Soil solarisation is a cultural method for reducing the inoculum potential of soil-borne pathogens. Moist soil is covered with transparent polyethylene sheeting and heated by solar energy for several days or weeks during the hottest time of the year. Katan (1980) states that there are four requirements for successful solarisation. These are:

i) polyethylene mulching of the soil has to be completed before planting
ii) soil must be kept wet during mulching to improve thermal conduction and increase the heat sensitivity of resting propagules
iii) the time required to control pathogens in the lower levels of the soil profile is longer than that needed for surface soil,
iv) thin transparent polyethylene is used because it is cheap, yet effective.

Soil temperatures fluctuate during soil solarisation. Stapleton (2000), working in open fields in California, reported soil temperatures in a diurnal range of 50/37^{0}C at 10 cm and 43/37^{0}C at 20 cm with a 35/20^{0}C air temperature. This is considerably higher than values obtained by Galati and McKay (1996) for carrot fields north of Perth. They recorded a diurnal range of 40/20^{0}C at 10 cm and 36/25^{0}C at 20 cm with a 40/15^{0}C air temperature.

One way to determine whether solarisation has potential for controlling pathogens is by *in vitro* experiments on the survival of resting spores exposed to a range of temperatures for different lengths of time. We have used this method to determine the survival of *Pythium sulcatum* and *P. violae* at different combinations of temperature and time.

Materials and methods

Hyphal tip culture of *Pythium sulcatum* and *P. violae* were grown on potato carrot agar (Plaats-Niterink, 1981) at 20^{0}C for at least 8 weeks, to allow oospores and other resistant propagules to develop. The cultures used are shown in Table 27. Agar discs, 5 mm diameter, were punched out of each test culture. A minimum of 20 discs were wrapped in a piece of sterile Miracloth (Calbiochem-Novabiochem Corporation, La Jolla, CA 92039, USA) for ease of handling, for each time x temperature combination.

Table 27. Isolates used in the temperature experiments.

Identity	WAC number	State	Presence of oospores and hyphal swellings
P. sulcatum	WAC8470	WA	Oospores, hyphae
P. sulcatum	WAC9654	NSW	Hyphae only
P. sulcatum	WAC9666	SA (MR)	Oospores, hyphal swellings, hyphae
P. sulcatum	WAC9669	QLD	Hyphal swellings, hyphae
P. sulcatum	WAC9673	TAS	Oospores, hyphae
P. sulcatum	WAC9687	VIC	Oospores, hyphal swellings, hyphae
P. sulcatum	WAC9690	NSW	Oospores, hyphal swellings, hyphae
P. sulcatum	WAC9696	VIC (MR)	Oospores, hyphal swellings, hyphae
P. sulcatum	WAC9701	QLD	Oospores, hyphal swellings, hyphae
P. violae	WAC9651	VIC (MR)	Oospores, hyphae
P. violae	WAC9668	SA (MR)	Oospores, hyphal swellings, hyphae
P. violae	WAC9695	VIC (MR)	Oospores, hyphae

McCartney bottles containing 20 ml sterile water were left to equilibrate for at least 2 hr. at the appropriate temperature, in a GFL waterbath (GFL Gesellschaft für Labortechnik mbH, Schulze – Delitzsch – Strasse 4, D-30938 Burgwedel – Germany). The agar discs, supported on the Miracloth, were plunged into the bottles of sterile water and incubated for the appropriate time. There was a separate McCartney bottle for each isolate x time x temperature combination. Discs incubated for 0 hr. were immersed in water at the appropriate temperature for 15 secs. After adding the agar discs, the McCartney bottles were returned to the waterbath. Initial tests were carried out at 30 and 40^{0}C, for 0, 1, 2, 6, 24 and 48 hr. Additional time x temperature combinations depended on the results from this initial experiment.

After the appropriate incubation time the Miracloth containing the agar discs was removed from each McCartney bottle and 20 discs plated onto *Pythium* selective agar (see section 3.1). The plates were incubated at 20^{0}C and examined after 5, 8 and 12 days.

Survival is defined as the proportion of discs from which the test fungi grew.

Results

P. sulcatum

The survival of *P. sulcatum* varied between isolates for different time x temperature combinations, so not all isolates were tested at all temperatures. Detailed results are given in Table 28.

Table 28. Survival of *Pythium sulcatum* isolates at different time x temperature combinations. Proportion (%) of inoculum discs from which *P. sulcatum* grew after incubation in water for different times.

Isolate	Temperature (°C)	Time (hr) 0	1	2	6	24	48
WAC8470	30.0	100	100	100	100	100	85
	37.5	100	100	100	100	100	10
	40.0	100	100	100	100	0	0
	42.5	100	100	100	100	0	n/t
WAC9654	30.0	100	100	100	100	100	100
	35.0	100	100	100	100	0	n/t
	37.5	100	100	95	5	0	0
	40.0	100	0	0	0	0	0
WAC9666	30.0	100	100	100	100	100	100
	35.0	100	100	100	100	100	n/t
	37.5	100	100	100	100	70	35
	40.0	100	60	45	40	0	0
	42.5	100	65	20	0	0	n/t
WAC9669	30.0	100	100	100	100	100	100
	35.0	100	100	100	100	100	65
	37.5	100	100	95	0	0	0
	40.0	100	0	0	0	0	0
WAC9673	30.0	100	100	100	100	100	100
	37.5	100	n/t	n/t	100	100	100
	40.0	100	100	100	100	30	0
	42.5	100	100	100	0	0	n/t
	45.0	100	100	85	0	n/t	n/t
WAC9687	30.0	100	100	100	100	100	100
	35.0	100	100	100	100	100	100
	37.5	100	100	100	90	0	0
	40.0	100	0	0	0	0	0
WAC9690	30.0	100	100	100	100	100	100
	35.0	100	100	100	100	100	55
	37.5	100	100	100	80	0	0
	40.0	100	55	15	0	0	0
	42.5	100	25	5	0	0	n/t
WAC9701	30.0	100	100	100	100	100	100
	37.5	100	100	100	100	0	0
	40.0	100	5	5	0	0	0
WAC9696	30.0	100	100	100	100	100	100
	37.5	100	n/t	n/t	100	100	0
	40.0	100	100	100	100	0	0
	42.5	100	100	100	100	0	n/t

n/t: not tested

Individual results have been combined to give the survival of *P. sulcatum* isolates at the different time x temperature combinations tested (Table 29).

Table 29. Survival of *P. sulcatum* isolates at different time x temperatue combinations; +: growth within 12 days, -: no growth within 12 days.

Temperature (°C)	Time (hr) 0	1	2	6	24	48
30.0	+	+	+	+	+	+
35.0	+	+	+	+	+	+
37.5	+	+	+	+	+	+
40.0	+	+	+	+	+	-
42.5	+	+	+	+	-	n/t
45.0	+	+	+	-	n/t	n/t

n/t: not tested

P. violae

The survival of *P. violae* varied between isolates for different time x temperature combinations. Detailed results are given in Table 30.

Individual results have been combined to give the survival of *P. violae* isolates at the different time x temperature combinations tested (Table 31).

Table 30. Survival of *Pythium violae* isolates at different time x temperature combinations. Proportion (%) of inoculum discs from which *P. violae* grew after incubation in water for different times.

Isolate	Temperature (°C)	Time (hr) 0	1	2	6	24	48
WAC9651	30.0	100	100	100	100	100	90
	32.5	100	100	100	100	15	0
	35.0	100	0	0	0	0	0
	40.0	100	0	0	0	0	0
WAC9668	30.0	100	100	100	15	5	0
	32.5	100	100	100	100	10	10
	35.0	100	0	0	0	0	0
	40.0	45	0	0	0	0	0
WAC9695	30.0	100	100	100	100	90	55
	32.5	100	100	100	100	0	0
	35.0	100	5	0	0	0	0
	40.0	85	0	0	0	0	0

Table 31. Survival of *P. violae* isolates at different time x temperature combinations: +: growth within 12 days, -: no growth within 12 days.

Temperature (°C)	Time (hr) 0	1	2	6	24	48
30.0	+	+	+	+	+	+
32.5	+	+	+	+	+	+
35.0	+	+	-	-	-	-
40.0	+	-	-	-	-	-

P. sulcatum survived for longer, and at higher temperatures, than *P. violae* (Table 29, Table 31).

Discussion

If solarisation is to be an effective method of controlling cavity spot disease it must eliminate, or greatly reduce the number of infective propagules of *Pythium sulcatum* and/or *P. violae* in soil. Control must occur at a depth of at least 15 cm because export quality carrots must be at least 150 mm long.

Our *in vitro* experimental results show that *P. sulcatum* survives at higher temperatures than *P. violae* (Table 29, Table 31). Isolates of *P. violae* failed to survive for 2 hr. at 35°C while one *P. sulcatum* isolate survived for 2 hr. at 45°C, and two isolates survived for 6 hr. at 42.5°C (Table 28, Table 30).

The temperatures achieved by Galati and McKay (1996) would be insufficient to reduce survival of *P. sulcatum* in Western Australia, although they would probably be sufficient to reduce the inoculum potential of *P. violae*. It might be possible to improve the efficacy of solarisation by using thermal-infrared absorbing film (Chase *et al.*, 1999), applying it for longer and carrying solarisation out during January and February.

Another method for heating field soil is by using a self-propelled, soil-steaming machine. Pinel *et al.* (2000) showed that this method killed pathogens and weed seeds in the top 15 cm of light soil, but failed to control a range of fungal pathogens at 20 cm depth.

Section 4. Chemical control

E. M. Davison, A. G. McKay and P. A. Murphy

4.1 Chemical and microbial control of cavity spot (00md15)

Summary. A range of chemical (acibenzolar-S-methyl, azoxystrobin, didecydimethyl-ammonium chloride, dimethomorph, fluazinam, flusulfamide, metalaxyl, phosphorus acid, propamocarb) and microbial (SC27®, Trichoflow™-T, E-2001®, EM microbes®) formulations were applied either before planting or during the growth of carrots, cv Ivor, on a site infested with *Pythium sulcatum*, the cause of cavity spot disease. Seedling harvests of 7 or 8 week old plants showed that *Pythium* infection was only reduced in the metalaxyl treatment. At the final harvest, 17 weeks after seeding, there was no significant ($P<0.05$) reduction in the incidence or severity of cavity spot in any treatment. Soil application of flusulfamide before planting resulted in a large proportion of forked carrots.

Introduction

Although the systemic fungicide metalaxyl is used to control *Pythium* diseases of carrots (Lyshol *et al.*, 1984; Wheatley *et al.*, 1984; Walker, 1988; Sweet *et al.*, 1989; Walker, 1991), it becomes less effective with repeated use. Davison and McKay (1999) found that its failure to control these diseases on sites with a history of usage was associated with reduced persistence not reduced sensitivity of the target fungi. Authors from many regions have reported that enhanced breakdown of metalaxyl and other soil-applied chemicals is a result of enhanced microbial degradation (eg Bailey and Coffey, 1985; Droby and Coffey, 1991; Stirling *et al.*, 1992; Walker, 1993; Warton and Matthiessen, 2000). It is probably a widespread, but unrecognised problem in many horticultural soils.

One way to reduce the risk of enhance breakdown occurring is to use a range of chemicals from different chemical groups for pest and disease control. At the present time, metalaxyl, as Ridomil Gold® 25SG, is the only chemical registered for controlling *Pythium* spp. and *Phytophthora* spp. in carrots in Australia. Other fungicides that have activity against this group of fungi include azoxystrobin (Wong and Wilcox, 2000), dimethomorph (Powelson and Inglis, 1999), phosphorous acid (Walker, 1988) and propamocarb (Cohen and Coffey, 1986). Another way of reducing the impact of soilborne diseases is to treat the soil before seeding, rather than treating the growing crop. Chemicals that might be suitable for pre-plant treatment include fluazinam (Matheron and Porchas, 2000), flusulfamide (Tanaka *et al.*, 1999) and didecyldimethylammonium chloride. Some chemicals, such as acibenzolar-S-methyl, that are applied to plants act indirectly by activating plant defense response (Matheron and Porchas, 1999).

Biological control of soilborne pathogens is another option. *Trichoderma harzianum* preparations that suppress *Pythium* spp. are commercially available (Harman, 2000). Other microbiological preparations that are sold as soil conditioners, may have some efficacy in reducing root diseases.

In this experiment we have compared the ability of a range of chemical and biological products to control *Pythium* diseases of carrots on a site with a high level of cavity spot. The rate(s) and time(s) of application were decided in consultation with the manufacturers or distributors of the various products. The results are given below.

Materials and methods

Site description and experimental design

The experiment was located on a 24 x 100 m site that had been infested with cavity spot in 1998, in the East block, Medina Research Station, and subsequently cropped with the susceptible cultivar Ivor. A survey of the incidence of cavity spot in the previous mature carrot crop, was conducted in December 1999. Twelve carrots were assessed for cavity spot and forking at 10 m interval down each bed. This survey showed that the incidence of cavity spot varied between beds, but did not vary along the beds (Table 32). As a consequence of this survey, beds 4, 5 and 6 were used for the experiment.

The soil type is Karrakatta sand. The pH of the adjacent site varied between 6.5 and 7.2 in water (5.8 to6.6 in 0.01 M $CaCl_2$).

The experiment was a randomised block with 15 treatments, replicated four times. Experimental plots were one, 1.5 m raised bed wide, by 6 m long.

Table 32. Grid survey of the incidence of cavity spot disease in carrots harvested in December 1999.

Bed		Distance along the bed	
Number	Incidence of cavity spot (%)	Distance (m)	Incidence of cavity spot (%)
1	49	5	58
2	58	15	85
3	61	25	71
4	81	35	61
5	84	45	71
6	84	55	68
7	84	65	68
		75	82
		85	76
		95	76
Significance	***		n.s.
LSD (P<0.05)	15		

*** = P< 0.001, ** = P< 0.01, * = P< 0.05, n.s. = P>0.05

Site preparation

Irrigation was by knocker sprinklers at 12 x 9 m, operating at more than 30 psi. The site was cultivated with deep tynes before the beds were formed. As two treatments (9 and 11) needed to be applied 2 weeks before seeding, the beds were formed and plots marked, and then reformed and plots remarked immediately before seeding.

Agran (ammonium nitrate) at the rate of 100 kg ha^{-1}, was applied 2 weeks before seeding and watered in with 50 per cent daily pan evaporation. Immediately before seeding, basal fertiliser of 1,500 kg ha^{-1} superphosphate and 150 kg ha^{-1} standard trace element mix were broadcast onto moist soil. Post-planting fertiliser was applied in a fertigation program that commenced 2 weeks after seeding. It supplied a total of 282 kg N ha^{-1}, 288 kg P ha^{-1}, 18 kg Mg ha^{-1} and 1 kg B ha^{-1} to the growing crop.

Carrot seed cultivar Ivor, was sown on 3 October 2000, in four double rows per 1.5 m wide bed, with a target density of 60 plants m^{-2}. Post-planting weed control was by applying a tank mix of 2L ha^{-1} trifluralin and 1L ha^{-1} Afalon®, 2 days after seeding. Fusilade® and/or linuron (Afalon®) (at 1.1 L ha^{-1}) were used for post-emergence weed control after the three true-leaf stage.

Treatments

The treatments applied are shown in Table 33. Unless otherwise stated, the compounds were applied onto soil or plants with a boom spray with fan nozzles. The compounds were applied twice, at half rate, to each plot to ensure even application.

Table 33 Chemical and microbial treatments used to control cavity spot and related *Pythium* diseases.

Treatment	Product type	Active ingredient	Trade name®	Supplier
1	Control			
2	Control			
3	Chemical	Fluazinam	Shirlan®	Crop Care Australasia Pty Ltd
4	Chemical	Flusulfamide	Nebijin®	Mitsui Toatsu Chemicals Inc.
5	Chemical	Metalaxyl	Apron®	Novartis Crop Protection Pty Limited
6	Chemical	Acibenzolar-S-methyl	Bion®	Novartis Crop Protection Pty Limited
7	Biological		SC27®	CLM
8	Biological	*Trichoderma*	Trichoflow™-T	Lefroy Valley
9	Biological		E-2001®	E-2001 W.A.
10	Biological		EM microbes®	EM Distributors WA
11	Chemical	Didecyldimethylammonium chloride	Sporekill™	Hygrotech Oceania
12	Chemical	Azoxystrobin	Amistar® WG	Crop Care Australasia Pty Ltd
13	Chemical	Propamocarb hydrochloride	Previcur®	Hoechst Schering Agrevo Pty Ltd
14	Chemical	Dimethomorph + mancozeb	Acrobat® MZ 690	Cyanamid Agriculture Pty. Limited
15	Chemical	Phosphorous acid	Fos ject® 200	UIM Agrochemicals, Unitec Group Pty Ltd

Treatments 1 and 2. Control. No treatments to control root disease.

Treatment 3, Shirlan®. Fluazinam, as Shirlan®, was sprayed onto at the rate of 1.5 kg a.i. ha^{-1}, 1 day before seeding. It was watered in after application.

Treatment 4, Nebijin®. Flusulfamide, as Nebijin®, was applied at the rate of 0.9 kg a.i. ha^{-1}, 1 day before seeding. It was watered in after application.

Treatment 5, Apron®. Metalaxyl, as Apron®, was applied at the rate of 3 kg a.i. ha^{-1}, 6 weeks after seeding.

Treatment 6, Bion®. Acibenzolar-S-methyl, as Bion®, was applied at the rate of 0.05 kg a.i. ha^{-1}, 6 weeks after seeding.

Treatment 7, SC27®. SC27® was applied at the rate of 1 L ha^{-1}, at 1, 5, 9 and 13 weeks after seeding.

Treatment 8, Trichoflow™-T. Trichoderma, as Trichoflow™-T plus Cittowett®, was applied at the rate of 10 kg ha^{-1}, immediately after seeding, and at 3, 6, 9 and 13 weeks after seeding.

Treatment 9, E-2001®. E-2001® was applied at the rate of 100 L ha^{-1}, 2 weeks before seeding. The E-2001 was prepared by adding 40 ml E-2001 stock solution (provided by the supplier), plus 100 ml Multibacter®, to 4 L autoclaved tapwater. This solution was incubated at 24^0C for 4 days, and then diluted with an equal volume of autoclaved tap water. This half strength

solution was sprayed twice onto the test plots, followed by cobalt chloride at 10 g in 4 L water.

Treatment 10, EM microbes®. EM microbes®, at 10 L ha^{-1}, were applied to the plots 1 week before seeding, and then 1 day before seeding.

Treatment 11, Sporekill™. Didecyldimethylammonium chloride, as Sporekill™, was applied at the rate of 1 L a.i. ha^{-1}, 2 weeks before seeding.

Treatment 12, Amistar®. Azoxystrobin, as Amistar®, was applied at the rate of 0.4 kg a.i. ha^{-1}, 1 day before, and 6 and 12 weeks after, seeding.

Treatment 13, Previcur®. Propamicarb, as Previcur®, was applied at the rate of 3.6 kg a.i. ha^{-1} after seeding.

Treatment 14, Acrobat®. Dimethomorph, as Acrobat®, was applied at the rate of 2 kg a.i. ha^{-1}, at 5, 9 and 13 weeks after seeding.

Treatment 15, Fos ject®. Phosphorous acid, as Fos ject®, was applied at the rate of 12 L a.i. ha^{-1}, at 5, 9 and 13 weeks after seeding.

Seedling harvest

Because of the large number of treatments, there were two seedling harvests. The first harvest was 7 weeks after seeding (21 November 2001) and comprised Treatments 1, 4, 7, 8, 9, 10 and 11. The second harvest was 7 days later, and comprised Treatments 2, 3, 5, 6, 12, 13, 14 and 15. At each harvest a linear sample of 20 seedlings was taken from one of the middle two rows of carrots in each plot. The roots were washed thoroughly, and examined for lesions. Each tap root was cut into 1 cm pieces, and five pieces plated directly onto agar selective for *Pythium* (section 3.1). All plates were incubated at room temperature. *Pythium* spp. growing from the roots were characterised on the basis of colony morphology, growth rate and morphology of the oogonia, antheridia and oospores.

Final harvest

The experiment was harvested on 23 January 2001. At harvest, three 1 m lengths of row from one of the middle rows of each plot were hand harvested, bulked in onion bags, washed, and then stored in polythene bags in a cold room at 1^0C until they were assessed.

The quality of the bulk crop was assessed by number and by weight into the following categories: export marketable (>150 mm long, 25-50 mm crown diameter), short marketable (120-150 mm long, 25-50 mm crown diameter), undersized (<120 mm long, or <25 mm crown diameter), oversize (>50 mm crown diameter), forked, misshapen, split. These categories were used for each cavity spot rating of 0, 1, 2, 3 and 4 or more spots.

Isolations were made from a random sample of 24 carrots. One spot from each carrot was plated onto agar selective for *Pythium*, and any isolates characterised as described above.

Statistical analysis

GENSTAT version 5 (1993) was used to analyse the results using transformed or untransformed data as appropriate.

The analysis of the incidence and severity of seedling infection was analysed using block as the block stratum, and treatment as the treatment stratum for each harvest time. For the final harvest, the incidence and severity of cavity spot was analysed using block as the block stratum, and treatment as the treatment stratum.

Results

Seedling harvests

Pythium spp., including *P. sulcatum*, were isolated from carrot seedlings in all treatments. There was no significant difference in the incidence and severity of infection in the first harvest, but in the second harvest there was significantly less infection in the metalaxyl treatment (Treatment 5) (Table 34).

Table 34 Incidence and severity of *Pythium* infection of carrot seedlings.

Treatment	Incidence of *Pythium* (%)	Incidence of *P. sulcatum* (%)	Severity of *Pythium* (%)	Severity of *P. sulcatum* (%)
Harvest 1, after 7 weeks				
1 Control	25	20	6.0	5.0
4 Nebijin®	18	13	3.5	2.5
7 SC27®	23	16	4.8	3.3
8 Trichoflow™-T	19	11	4.5	3.0
9 E-2001®	11	10	2.3	2.0
10 EM microbes®	25	23	6.3	5.3
11 Sporekill™	11	11	2.5	2.5
Significance	n.s.	n.s.	n.s.	n.s.
Harvest 2, after 8 weeks				
2 Control	25	25	6.3	6.3
3 Shirlan®	15	13	4.3	3.5
5 Apron®	5	5	1.3	1.3
6 Bion®	20	18	4.5	4.0
12 Amistar®	18	15	4.0	3.5
13 Previcur®	15	10	3.5	2.5
14 Acrobat®	15	14	4.0	3.8
15 Fos ject®	36	31	9.3	8.3
Significance	*	*	*	0.05
LSD (P<0.05)	16	15	4.2	

*** = P< 0.001, ** = P< 0.01, * = P< 0.05, n.s. = P>0.05

Final harvest

There was no difference in either the total weight or number of carrots harvest from the different treatments, although there was a significant difference in the export yield (Table 35, Table 36). There was a very high proportion of forked carrots in the Nebijin® treatment (Treatment 4).

There was no significant difference in the proportion of carrots with cavity spot (Table 35, Table 36). The only treatment that looked promising was Amistar®, which had the lowest incidence of cavity spot, the lowest number of spots per carrot, and the lowest proportion of carrots with four or more spots (Table 35, Table 36).

P. sulcatum was isolated from 33 per cent of the 24 lesions plated.

Table 35 Comparison of the number of mature carrots (cv Ivor), proportion of different grades of carrot irrespective of cavity spot, and proportion of carrots with cavity spot irrespective of grade.

Treatment	Total number	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)	Cavity spot 4 (%)	Cavity spots per carrot
1 Control	58.0	65.3	6.8	11.2	0.0	3.3	12.6	0.9	70.3	35.0	2.4
2 Control	57.8	55.5	5.8	6.5	0.0	3.3	27.9	1.0	76.5	50.4	3.0
3 Shirlan®	60.3	62.3	9.0	8.4	0.3	3.5	16.1	0.4	77.5	41.8	2.8
4 Nebijin®	62.0	34.6	3.8	4.7	0.0	35.6	19.3	2.0	77.2	45.3	2.8
5 Apron®	52.5	69.0	6.8	6.6	0.0	1.7	14.7	1.2	90.2	68.3	3.8
6 Bion®	63.7	62.5	5.7	8.4	0.8	1.4	19.6	1.6	81.5	39.1	2.7
7 SC27®	58.5	65.7	7.8	8.8	0.0	2.5	13.4	1.7	87.6	59.4	3.4
8 Trichoflow™-T	65.2	57.9	4.2	3.7	0.0	2.5	31.8	0.0	77.2	33.3	2.5
9 E-2001®	66.0	68.6	9.3	5.7	0.0	2.8	11.8	1.8	87.3	58.6	3.4
10 EM microbes®	55.0	69.2	10.2	5.1	0.0	1.8	11.6	2.1	84.4	54.2	3.3
11 Sporekill™	56.0	64.8	6.7	6.3	0.0	3.0	18.1	1.1	82.3	54.7	3.2
12 Amistar®	65.0	70.7	5.3	5.0	0.3	2.8	15.5	0.3	62.7	25.7	1.9
13 Previcur®	63.5	69.0	4.5	5.2	0.0	3.5	16.1	1.4	79.6	32.7	2.5
14 Acrobat®	64.5	56.5	5.5	4.1	0.0	1.8	30.4	1.6	84.1	49.3	3.1
15 Fos ject®	68.7	65.9	7.6	3.4	0.0	0.7	21.7	0.7	73.7	39.2	2.5
Significance		**	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.	0.06	0.12
LSD		14.7				3.1					

*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, n.s. = $P > 0.05$

Table 36 Comparison of the weight of mature carrots (cv Ivor), proportion of different grades of carrot irrespective of cavity spot, and proportion of carrots with cavity spot irrespective of grade.

Treatment	Total weight (kg)	Export yield (%)	Marketable short (%)	Undersize (%)	Oversize (%)	Forked (%)	Misshapen (%)	Split (%)	Cavity spot (%)	Cavity spot 4 (%)
1 Control	7.2	76.6	4.0	3.3	0.0	3.3	11.9	1.1	73.3	38.6
2 Control	7.1	64.9	3.5	2.4	0.0	3.2	23.9	2.1	80.0	55.0
3 Shirlan®	7.6	70.8	6.7	3.1	0.8	3.5	14.6	0.4	77.3	42.8
4 Nebijin®	7.4	40.7	2.9	1.1	0.0	36.7	16.4	2.2	77.3	48.2
5 Apron®	6.7	77.4	4.7	2.8	0.0	1.2	12.6	1.3	92.1	70.3
6 Bion®	8.2	71.1	3.7	3.0	1.8	1.7	16.6	2.2	83.5	41.5
7 SC27®	7.1	75.2	5.8	3.7	0.0	3.1	9.9	2.4	89.2	61.2
8 Trichoflow™-T	8.3	67.7	2.9	1.2	0.0	2.1	26.2	0.0	79.1	37.1
9 E-2001®	8.0	76.2	5.8	2.2	0.0	2.6	10.6	2.5	88.4	61.4
10 EM microbes®	7.0	78.3	6.6	1.8	0.0	1.9	9.7	1.7	86.4	57.5
11 Sporekill™	7.0	72.8	4.2	1.7	0.0	4.0	15.4	1.9	86.0	57.8
12 Amistar®	8.4	78.7	3.7	1.5	0.8	3.0	12.0	0.3	62.9	26.7
13 Previcur®	7.8	74.0	3.2	1.9	0.0	3.2	15.9	1.8	82.2	35.1
14 Acrobat®	7.9	69.3	3.4	1.7	0.0	1.5	22.5	1.6	85.2	52.3
15 Fos ject®	8.3	72.2	5.1	1.5	0.0	0.5	19.7	1.1	73.5	39.5
Significance		***	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.	0.07
LSD		14.4				3.1				

*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, n.s. = $P > 0.05$

Discussion

The only product that resulted in a significant reduction in *Pythium* infection was metalaxyl, as Apron®. It reduced the incidence and severity of seedling infection (Table 34), but did not reduce the incidence or severity of cavity spot at the final harvest.

Azoxystrobin, as Amistar®, showed the lowest incidence of cavity spot, the lowest incidence of carrots with four or more spots, and the smallest number of spots per carrot (Table 35, Table 36) and would be worth further experimentation in terms of rate(s) and application time(s).

The chemical pre-plant treatments did not reduce the incidence of *Pythium* diseases. Flusulfamide, as Nebijin®, must have damaged seedling taproots, because of the high incidence of forked carrots at the final harvest (Table 35, Table 36).

None of the biological agents used in this experiment showed any efficacy against seedling infection, cavity spot or forking (Table 34, Table 35, Table 36)

The results from this experiment have been disappointing because it has indicated only one additional chemical or biological treatments that has the potential to minimise *Pythium* diseases of carrots.

4.2 Survey of the incidence of enhanced breakdown of metalaxyl on carrot farms

Summary A survey was carried out to determine whether there was evidence of enhanced breakdown of the fungicide metalaxyl on sites where it has been used in the past. Metalaxyl was added to soil samples from carrot properties in South Australia, Tasmania and Western Australia and the half-life determined by chemical analysis. The half-life varied from less than 1 day to 43 days, compared with a published value of 70 days. Enhanced breakdown of metalaxyl is a widespread and developing problem.

Introduction

The fungicide metalaxyl controls cavity spot and other *Pythium* diseases of carrots when applied at, or shortly after seeding, or to the growing crop (Lyshol *et al.*, 1984; Wheatley *et al.*, 1984). Davison and McKay (1999), however, found that it failed to control these diseases on properties with a history of use. The half-life of metalaxyl in soil is about 10 weeks (Bailey and Coffey, 1985; Kookana *et al.*, 1995), however, in soil where it did not control cavity spot the half-life was 10 days or less (Davison and McKay, 1999). Metalaxyl is broken down by soil microorganisms, and poor persistence is probably the result of enhanced biodegradation (Bailey and Coffey, 1985; Droby and Coffey, 1991).

We report a survey of soil from carrot properties to determine whether enhanced breakdown of metalaxyl is widespread.

Materials and methods

Soil sampling

About 1 kg of a bulked soil sample was taken from the headland on the up-wind side of a carrot field, and three additional samples were taken from the field itself. Each sample was composed of at least 24 sub-samples taken with a sterile scoop, with a separate scoop being used for each sample. The samples were forwarded to the WA Department of Agriculture.

Determination of the half-life of metalaxyl

Care was taken to ensure that there was no cross contamination between the soil samples. Each soil sample was weighed and moisture content determined.

0.5 ml of a 0.857 per cent solution of Apron® was added to the equivalent weight of 500 g oven dry soil. The soil sample was sieved six times through a sterile 3 mm sieve to ensure thorough mixing. A 120 g sub-sample was removed for analysis, and the remaining soil placed in a sterile, wide-necked lidded jar. A hole in the lid was sealed with filter paper to allow gas exchange. The jars were stored at 22^{0}C and sub-samples taken after 2 and 4 weeks. All sub-samples were stored at –20^{0}C before their metalaxyl content was determined.

Metalaxyl content of the soil was determined by acetone extraction followed by dual capillary column gas chromatography with thermionic detection (Calverly and Unwin, 1981).

Statistical analysis

The proportion of metalaxyl remaining in the soil was calculated for each harvest time. An exponential curve was fitted to these values using GENSTAT. The half-life was calculated using the formula:

half-life = -(1/C)*LN((T0/2-A)/B

where A, B and R are values from the fitted curve A+B*R**X,
C is (-LN(R)),
and T0 is (A+B).

Results

A total of 44 samples were tested from carrot properties in South Australia, Tasmania and Western Australia. The half-life varied from less than 1 day to 43 days (Table 37).

Table 37. Half-life of metalaxyl in soil samples from carrot properties.

State	Post code	Number of samples	Half-life (days)
SA	5355	2	28-29
SA	5341	5	21-23
TAS	7306	4	nd
TAS	7310	20	6-24
WA	6065	4	28-30
WA	6167	1	30
WA	6171	8	<1-43
Total		44	<1-43

nd: half-life could not be determined.

Discussion

The half-life of metalaxyl is 70 days in soil that has not been previously treated with metalaxyl (Bailey and Coffey, 1985; Kookana *et al.*, 1995). The half-life of all of the sampled soils (Table 37) is less than 70 days. These results indicate that enhanced degradation is occurring on many carrot growing properties in Australia, and is severe on some sites. Metalaxyl is not a long-term solution to controlling cavity spot and other *Pythium* diseases.

Section 5. Carrot variety screening for cavity spot tolerance

Allan McKay, Elaine Davison and Robyn Brett[10]

5.1 Carrot variety screening for cavity spot tolerance - 1999 to 2001 plantings (94MD32)

Summary. Identification of carrot varieties tolerant to cavity spot, that are also suitable for export production, is an important part of integrated disease control. Between 1999 and 2001 further variety screening was carried out in a cavity spot disease nursery at the Medina Research Station in Western Australia. Three farm trials were also planted in Victoria to confirm the relative cavity spot tolerance of varieties. Many of the most cavity spot tolerant varieties identified did not produce the high root quality demanded by export markets. The variety Stefano combines the characters of moderate yield and cavity spot tolerance with high root quality. Stefano has become established as the industry standard variety throughout Australia.

Introduction

Varietal tolerance to cavity spot is an important component of cavity spot disease management. Our aim has been to screen carrot varieties, mainly Nantes types, for tolerance to cavity spot. *Pythium sulcatum* is the causal organism of cavity spot in Western Australia (Davison and McKay, 1998) and has also been isolated from diseased carrots grown on the Mornington Peninsular and the Hills region east of Melbourne. *P. violae* has been found associated with cavity spot of carrots grown along the Murray River in northern Victoria.

The disease nursery established at Medina Research Station in 1994 was used to screen carrot varieties for tolerance to cavity spot from four plantings as part of a previous HRDC project VG 95010 (McKay and Davison, 2000). In a continuation of this work, carrot varieties were assessed for cavity spot tolerance, yield and quality from a further three plantings in the disease nursery at Medina Research Station. Three carrot variety trials were also planted on commercial carrot farms in Victoria with the aim of confirming the relative cavity spot tolerance of a range of carrot varieties.

Materials and methods

Medina Research Station site

A cavity spot disease nursery site was established at Medina Research Station to enable screening of carrot varieties under high disease pressure. In 1994 the site was inoculated with cavity spot infected carrots from a commercial crop, which were spread over the site and rotary hoed in. The cavity spot susceptible variety Primo was then sown on the site. Following this crop, which developed moderate levels of cavity spot, variety plantings were established on one quarter of the site. The remainder of the site was resown to Primo to maintain a high disease inoculum. Thereafter the site was continuously cropped (usually only one crop per year) with Primo while the variety plantings (one quarter of site) were rotated around the site and were preceded by at least two bulk crops of Primo to limit variation in disease history.

[10] Institute for Horticultural Development, Agriculture Victoria, Private Bag 15, South East Mail Centre, Vic. 3180

Assessments of the distribution of cavity spot in the bulk Primo crops on the site showed that background disease levels were relatively uniform.

Pythium sulcatum has been identified as the cause of cavity spot on this site. Varieties included in the 1999, 2000 and 2001 plantings are shown in Table 38. Three varieties, the cavity spot tolerant variety Bolero, the susceptible variety Ivor and the highly susceptible variety Primo were included as control varieties in all plantings.

The site was deep cultivated with tynes before forming beds with a rotary hoe. Fertiliser applied and incorporated before planting 1,200 kg ha^{-1} superphosphate, 100 kg ha^{-1} potassium sulphate, 100 kg ha^{-1} ammonium nitrate and a trace element mix. In crop applications of nitrogen, potassium and magnesium were applied through the irrigation as sulphate of ammonia, potassium nitrate and magnesium sulphate. Total crop applications were close to 350 kg N ha^{-1}, between 330 and 360 kg K ha^{-1} and 20 Mg kg ha^{-1}. Two post-planting applications of 5 kg ha^{-1} of borax were also applied. Fenamiphos (Nemacur®) was also sprayed and incorporated prior to planting to control nematodes.

Table 38. Details of carrot variety plantings at Medina Research Station from 1999 to 2001 (+ sown in planting, - not sown).

			Planting 6	Planting 7	Planting 8
Sowing date			20 Dec 99	18 May 00	13 Feb 01
Harvest date – Early harvest			6 Apr 00	17 Oct 00	6 Jun 01
Late harvest			27 Apr 00	7 Nov 00	27 Jun 01
Crop age at harvest (days) – Early harvest			108	152	113
Late harvest			129	173	134
Variety	*Seed company*	*Type*			
Bolero	Vilmorin	Nantes	+	+	+
Bristol	Bejo	Nantes	-	+	+
Crusader	South Pacific	Nantes	+	+	-
F2499	Bejo	Nantes	-	-	+
Havana	Novartis	Nantes	+	+	+
Ivor	Lefroy Valley	Nantes	+	+	+
Joan	Henderson	Nantes	-	-	+
Kendo	South Pacific	Nantesx	+	+	+
Mojo	Novartis	Nantesx	-	+	+
Murdoch	South Pacific	Nantes	+	+	+
Nairobi	Bejo	Nantes	+	-	-
Nandor	South Pacific	Nantes	+	-	-
Navarre	Bejo	Nantes	+	+	+
Nepal	Bejo	Nantes	-	+	+
Nigel	Bejo	Nantes	-	+	+
Ostende	Henderson	Nantes	+	+	-
Primo	Vilmorin	Nantes	+	+	+
Ricardo	South Pacific	Nantes	-	+	-
Senator	Lefroy Valley	Nantes	-	-	+
Senior	Lefroy Valley	Nantes	+	+	-
Stefano	South Pacific	Nantes	+	+	+
Sunstar	Lefroy Valley	Nantes	-	-	+
Tempo	South Pacific	Nantes	+	+	-
Trevor	Lefroy Valley	Nantes	-	-	+
Virginia	Henderson	Nantes	-	-	+
Y3078	Yates	Nantes	+	+	-

Four double rows of the test varieties were seeded on beds 1.5 m wide with an Earthway® seeder. Seeding dates are shown in Table 38. Experimental plots were 3 m long, with four replicates. Emerged seedlings were thinned at the 2-leaf stage to give a target density of 70 plants/m^2. Plants were watered from overhead impact sprinklers. Irrigation was scheduled on Class A pan evaporation (Epan) with 0.8 of Epan applied until the 3-leaf stage, 1.0 Epan from the 3- to 7-leaf stage, 1.2 Epan from 7-leaf stage until full ground cover, and then 1.5 Epan until harvest. Linuron (Afalon®) and trifluralin (Treflan®) were used for pre-emergence weed control, while Afalon® and fluazifop-butyl (Fusilade®) were used as post-emergence herbicides.

Chlorothalonil based fungicides were applied if blight (*Alternaria dauci*) became established in foliage.

Plots were sampled at an early and a late harvest at the dates shown in Table 38. The quality of the varieties was assessed at harvest by weight into the following categories: marketable (>120 mm long, >25 crown diameter), undersize (<120 mm

long, or <25 mm crown diameter), forked, misshapen, split. These categories were used for each cavity spot rating of 0, 1, 2, 3 and 4 or more spots. Roots with three or more lesions were classed as having severe cavity spot. When grading Planting 8 all varieties were given an overall root quality score that reflected root smoothness on a rating scale of 1 (extremely rough) to 9 (ultra smooth).

On farm trials - Victoria

Three trials, one near Robinvale on the Murray River, one on the Mornington peninsular and one near Dandenong, were planted in commercial carrot fields in spring 2000. Ten carrot varieties (Table 42), with a range of cavity spot tolerances as measured under WA conditions were planted at each site. The planting near Robinvale was flooded soon after seeding and was subsequently abandoned.

Site 2 near Dandenong was harvested on 5 February 2001 and site 1 on the Mornington Peninsular was harvested the following day. At these two sites, varietal plots were unreplicated. Roots from two by 1 m lengths of row from each plot were harvested by hand and washed then coolstored before being graded according to the number of cavity spot lesions. All marketable yields reported here are calculated without accounting for cavity spot.

Results

Medina Research Station site

Severe cavity spot, defined here as three or more spots per carrot, gave a better separation of carrot varieties than total incidence, and these data are shown in Table 39.

The tolerant control variety Bolero had consistently lower incidence of severe cavity spot than the susceptible varieties Ivor and Primo (Table 39). In Planting 7, the variety by harvest time interaction for severe cavity spot incidence was significant while in Plantings 6 and 8 this interaction was not significant (Table 39).

Table 39. The incidence (proportion (%) of total yield) of severe cavity spot symptoms in carrot varieties from three plantings at Medina Research Station.

Variety	Planting 6		Planting 7		Planting 8	
	Early harvest	Late harvest	Early harvest	Late harvest	Early harvest	Late harvest
Bolero	16.9	54.5	8.0	35.2	0.8	0.8
Bristol			5.4	27.8	3.0	1.9
Crusader	39.7	75.9	5.0	62.5		
F2499					2.8	0.3
Havana	24.8	35.9	7.0	29.6	11.3	4.6
Ivor	72.4	87.6	15.5	44.9	5.8	5.9
Joan					4.0	3.0
Kendo	10.2	29.5	17.6	45.8	1.2	1.2
Mojo			3.6	68.3	8.2	1.7
Murdoch	53.0	85.6	34.3	71.3	8.1	13.2
Nairobi	36.9	31.2				
Nandor	12.8	47.5				
Navarre	3.5	35.3	8.3	18.1	4.3	1.5
Nepal			7.6	21.6	4.2	1.0
Nigel			12.4	33.3	2.0	2.8
Ostende	44.3	38.6	8.9	39.1		
Primo	72.6	95.0	33.1	78.5	13.0	11.7
Ricardo			12.4	66.0		
Senator					2.4	1.5
Senior	34.3	49.2	13.7	31.2		
Stefano	31.6	30.2	18.2	44.7	6.0	1.7
Sunstar					5.6	2.1
Tempo	68.1	92.9	10.4	45.4		
Trevor					1.8	0.0
Virginia					29.7	21.0
Y3078	10.4	20.5	16.9	28.8		
Mean	*35.4*	*54.0*	*13.2*	*44.0*	*6.3*	*4.2*
Variety (lsd P=0.05)	*P<0.001 (29.6)*		*P<0.001 (14.8)*		*P<0.001 (9.0)*	
Harvest (lsd P=0.05)	*P<0.001 (8.8)*		*P<0.001 (4.3)*		*P=0.077*	
Var x Har (lsd P=0.05)	*ns*		*P<0.001 (19.4)*		*Ns*	

Planting 6

At the first harvest, Murdoch, Tempo, Ivor and Primo had significantly higher levels of severe cavity spot than all other varieties (Table 39). At the second harvest three weeks later, Crusader, Murdoch, Tempo, Ivor and Primo ranked as the most susceptible to cavity spot. Among the most cavity spot tolerant varieties at the second harvest were Y3078, Kendo, Stefano, Nairobi and Navarre (Table 39). While the interaction between severe cavity spot incidence and harvest time was not significant in this planting, there was an indication of different rates of cavity spot development among varieties between harvests. For example, the level of severe cavity spot did not increase in Stefano and Nairobi between the two harvests, while it did increase rapidly in varieties such as Crusader and Nandor (Table 39).

Average total yield across all varieties increased from 54 to 68.5 t ha^{-1} in the 21 days between the first and second harvests (Table 40). Similarly average marketable yield increased from 38 to 49 t ha^{-1}. Nandor and Ivor (formerly Top Pak) had the highest total yields from the first harvest while Ivor had the highest total yield from the second harvest. Stefano and Kendo produced the smoothest roots and both showed good tolerance to cavity spot.

Planting 7

At the early harvest there was little separation between the varieties for cavity spot susceptibility. Only Primo and Murdoch had significantly more cavity spot than all other varieties at the early harvest (Table 39). At the late harvest more differences were apparent with more than a four-fold difference in severe symptoms between the most tolerant (Navarre) and most susceptible (Primo) varieties. The most cavity spot tolerant varieties included Navarre, Nepal, Bristol, Y3078, Havana, Senior, Nigel and Bolero. The most cavity spot susceptible varieties at the late harvest were Primo, Murdoch, Mojo, Ricardo and Crusader. The strong interaction between variety and harvest time for cavity spot is in contrast to the previous summer harvest where there was no variety by harvest time interaction for cavity spot incidence.

Mojo was quick to bulk up and produced a high yield of deep orange smooth roots from this autumn planting (Table 40). Cavity spot developed rapidly in 'over-mature' Mojo from the late harvest. There was also some concern over the brittleness of Mojo which may only be effectively tested by commercial harvesting and handling. Stefano produced high yields of good quality roots and although not amongst the most cavity spot tolerant varieties at the late harvest, it did produce superior quality. In the previous summer planting, Stefano produced consistently high quality roots and was amongst the most cavity spot tolerant varieties.

Total yield increased by an average of 11.4 per cent in the 21 days between the early and late harvests (Table 40) while during this period, marketable yields increased by only half this proportion (Table 41). Marketable proportion fell from an average of 73.8 per cent at the early harvest to 63.6 per cent at the late harvest. Independent of cavity spot, Mojo and Riccardo had the highest total yields at both the early and late harvests. Marketable yields of these varieties were also highest at both harvests but in this case were not significantly higher than several other varieties including Nigel, Kendo, Ivor and Murdoch.

The proportion of carrots classed as misshapen increased from 4.4 per cent at the early harvest to 10 per cent at the late harvest while growth splits increased from 1.1 per cent to 3.3 per cent between harvests. Murdoch (average 6.7 per cent) and Mojo (average 5.5 per cent) had the highest proportions of growth splits. Proportions of roots rejected for prominent eyes were low at this time of year being more of a problem in crop growing through summer. There were no significant differences among varieties or between harvests for proportion of forked roots which averaged 17.6 per cent of total yield.

Table 40. The total root yield (t ha^{-1}) of carrot varieties in three plantings at the Medina Research Station.

Variety	Planting 6		Planting 7		Planting 8	
	Early harvest	Late harvest	Early harvest	Late harvest	Early harvest	Late harvest
Bolero	54.6	69.9	73.0	83.5	77.2	89.2
Bristol			69.0	86.4	63.3	80.8
Crusader	50.1	65.3	79.9	98.1		
F2499					72.7	84.7
Havana	55.0	72.6	73.2	100.0	75.2	89.4
Ivor	65.0	81.6	88.0	106.3	79.5	95.7
Joan					76.2	92.7
Kendo	47.7	60.1	79.5	94.5	72.5	89.4
Mojo			92.1	117.9	89.8	112.9
Murdoch	50.3	68.7	84.5	92.8	87.5	102.6
Nairobi	55.5	77.1				
Nandor	66.2	68.4				
Navarre	58.9	75.1	80.0	102.2	69.7	89.1
Nepal			70.3	93.2	65.3	75.3
Nigel			89.3	110.2	76.4	92.0
Ostende	50.1	66.2	76.6	105.3		
Primo	50.0	55.2	84.3	105.1	79.2	89.7
Ricardo			100.8	119.6		
Senator					74.6	93.8
Senior	58.2	67.5	86.0	100.4		
Stefano	47.9	65.3	78.8	92.6	71.6	90.4
Sunstar					70.0	86.8
Tempo	53.6	71.9	80.9	105.5		
Trevor					75.9	87.9
Virginia					70.7	78.0
Y3078	52.7	62.4	71.9	88.9		
Mean	*54.4*	*68.5*	*81.0*	*100.2*	*74.8*	*90.0*
Variety	*P<0.001*		*P<0.001*		*P<0.001*	
(lsd P=0.05)	*(7.5)*		*(10.9)*		*(7.2)*	
Harvest	*P<0.001*		*P<0.001*		*P<0.001*	
(lsd P=0.05)	*(1.7)*		*(2.4)*		*(1.4)*	
Var x Har	*P<0.01*		*ns*		*P<0.051*	
(lsd P=0.05)	*(6.6)*				*(5.8)*	

Planting 8

Cavity spot incidence was very low in this planting possibly because harvests were in June. Cavity spot is less of a problem in Western Australia during the cooler winter months. Cavity spot incidence did not increase between the early and late harvests as is invariably the case in crops harvested during warmer weather (Table 39). Bolero had the lowest incidence of severe symptoms but only Murdoch, Primo and Virginia had significantly higher incidences (Table 39). From field observation, Virginia also appeared to be highly susceptible to leaf blight.

For this planting, average total yield increased from 74.8 to 90.0 t ha^{-1} while marketable yield increased from 53.2 to 61.2 t ha^{-1} in the 21 days between the early and late harvests (Table 40, Table 41). Mojo and Murdoch had the highest total and marketable yields from the early harvest (Table 40, Table 41). Nigel and Stefano had

the next highest marketable yields from the early harvest. Mojo had the highest total (112.9 t ha^{-1}) and marketable (98.3 t ha^{-1}) yields from the late harvest. Major causes of rejection from marketable grade were misshapen roots (average 20.1 per cent of total yield) and forking (average 5.2 per cent of total yield). Bulged eyes was also a source of root rejection and this disorder increased between the early (average 1.9 per cent) and late (average 4.1 per cent) harvests. Bolero and Bristol had the highest incidences of bulged eyes.

Root quality ratings are plotted in Figure 3. Difference among varieties for root quality ratings was significant ($P<0.001$) with Mojo, Kendo and Stefano rated as having the smoothest roots. Average rating fell ($P<0.01$) from 5.6 at the early harvest to 5.3 at the late harvest. There was no significant variety by harvest time interaction for root quality rating.

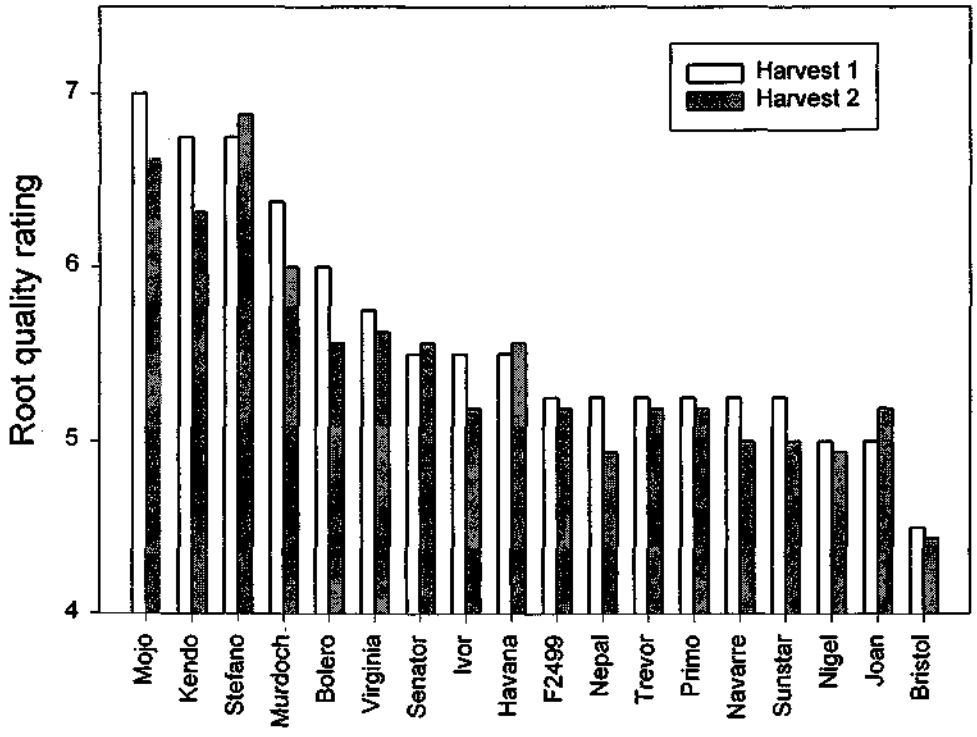

Figure 3. Root quality ratings for carrot varieties at the early (harvest 1) and late (harvest 2) harvests from planting 8. Rating scale is from 1 (extremely rough) to 9 (ultra smooth).

Table 41. Marketable yields (t ha^{-1}) of carrot varieties from three plantings at Medina Research Station.

Cavity spot has been ignored when calculating marketable yields.

Variety	Planting 6		Planting 7		Planting 8	
	Early harvest	Late harvest	Early harvest	Late harvest	Early harvest	Late harvest
Bolero	35.0	50.0	56.6	55.3	50.0	40.5
Bristol			45.6	44.3	34.6	40.3
Crusader	31.2	46.6	60.8	58.5		
F2499					49.2	65.0
Havana	40.6	59.7	51.5	68.2	55.1	59.5
Ivor	47.8	60.1	65.0	70.7	53.6	63.3
Joan					59.2	63.1
Kendo	37.0	48.7	68.2	70.2	49.4	63.9
Mojo			72.5	75.5	78.9	98.3
Murdoch	40.0	46.8	64.1	59.7	68.2	63.9
Nairobi	34.1	53.5				
Nandor	50.1	39.0				
Navarre	43.0	48.7	55.2	58.2	44.8	63.3
Nepal			48.7	59.8	40.2	45.7
Nigel			69.4	72.0	63.0	73.8
Ostende	39.0	54.2	60.4	63.4		
Primo	33.6	37.5	60.3	58.5	56.2	46.7
Ricardo			84.5	81.4		
Senator					44.7	67.6
Senior	39.7	47.5	63.7	64.4		
Stefano	31.5	50.7	63.0	69.7	60.5	74.9
Sunstar					40.3	53.5
Tempo	33.1	43.8	57.4	66.6		
Trevor					58.6	66.2
Virginia					50.8	52.1
Y3078	34.7	50.4	52.3	62.3		
Mean	*38.0*	*49.1*	*61.0*	*64.4*	*53.2*	*61.2*
Variety (lsd P=0.05)			*P<0.01 (15.4)*		*P<0.001 (13.6)*	
Harvest (lsd P=0.0)5			*P<0.05 (3.2)*		*P<0.001 (2.9)*	
Var x Har (lsd P=0.05)			*ns*		*P<0.01 (12.3)*	

On farm trials - Victoria

Average results from the two harvested sites are shown in Table 42. Carrots at site 1 were over-mature and carrots were large and quality was generally poor. Enlargement (bulging) of eyes was a common problem which was particularly severe in Havana and Y15009. Root quality would have been higher near normal commercial harvest, 3 weeks or so earlier. This late harvest would also have accounted for the 55 per cent higher average plot yield at site 1 compared to site 2 where carrots were approaching commercial maturity.

Table 42. Average total yields and cavity spot incidence and severity from two spring planted variety trials in Victoria.

Variety	Seed company	Ave yield (kg/plot)	Mean cavity spot incidence(%)	Ave 4+ cavity spot (%)
Havana	Novartis	4.9	5.0	0.0
Kendo	South Pacific	5.6	17.6	1.6
Ostende	Hendersons	4.5	1.7	1.7
Stefano	South Pacific	5.2	17.8	1.8
Navarre	Fairbanks	5.8	2.2	2.2
Crusader	South Pacific	4.0	13.7	2.9
Bristol	Fairbanks	5.0	15.3	3.3
Y15009	Yates	6.2	28.4	5.5
Ivor	Lefroy Valley	6.8	26.7	11.3
Murdoch	South Pacific	5.8	45.7	22.1

Moderate levels of cavity spot had developed at both sites. At site 1 the average cavity spot incidence across all varieties was nearly 20 per cent with an average 8 per cent of total yield having four or more lesions per root while at site 2 the corresponding figures were 15 and 2.6 per cent respectively. The most cavity spot tolerant varieties according to percentages of yield with four or more lesions per carrot were Havana, Kendo, Ostende, Stefano and Navarre. Both Keno and Stefano had over 17 per cent of yield with one to three lesions per root however the percentage of total root weight with four or more lesions was less than 2 per cent.

Discussion

The aim of this work is to identify Nantes, or Nantes cross varieties that produce high yields of export quality carrots that are tolerant to cavity spot disease. Very few of the varieties screened satisfy all of these requirements to be suitable for export carrot production under intensive carrot production.

This work has identified several varieties such as Mojo, Stefano and Kendo that produce moderate to high yields of high quality roots. Kendo and Stefano also combine these characters with moderate levels of field tolerance to cavity spot. The high yielding Mojo showed a propensity to develop cavity spot rapidly once marketable size was reached. Mojo may be more suitable for harvest during the cooler months in areas where cavity spot is caused by *Pythium sulcatum*. In these situations the risk of cavity spot losses could be minimized. In areas where the winter active *Pythium violae* causes cavity spot this strategy would not work.

In the farm trial in Victoria, Murdoch proved the most cavity spot susceptible variety at both sites as was expected from WA results. Greater variability is anticipated in unreplicated small plot trials however in general cavity tolerance rankings were as expected from replicated plantings in Western Australia. The failure of the replicated planting near the Murray River, where *P. violae* infection was anticipated, means the question of relevance of the carrot varietal tolerance rankings to *P. sulcatum* for areas with *P. violae* remains untested.

Acknowledgements

We thank Robyn Brett (Agriculture Victoria, Knoxfield), Robin Cole (South Australian Research and Development Institute, Adelaide), Sally-Ann Henderson (Agriculture Victoria, Irymple Research Station), Hoong Pung (Serve-Ag Research, Devonport, Tasmania), Rob O'Brien (Queensland Department of Primary Industry, Indooroopilly) and Len Tesoriero (New South Wales Agriculture, Camden) for providing *Pythium* cultures from the different carrot growing regions of Australia. We thank Giles Hardy (Murdoch University, Western Australia) and Geoff. White (Horticulture Research International, UK) for providing authenticated cultures of *P. sulcatum* and *P. violae*.

We thank J. Speijers for statistical advice.

We thank the following companies for the gift of chemical and biological agents and recommendations for their use: Crop Care Australasia Pty Ltd for the gift of Amistar® WG, Novartis Crop Protection Australasia Pty Limited for the gift of Bion® 50 WG, E-2001 W.A. for the gift of E-2001®, EM Distributors WA for the gift of EM microbes®, and Lefroy Valley for the gift of Trichoflow™-T.

We thank Paul Murphy, Rob Deyl, Tony Shimmin and Rohan Prince for technical assistance in Western Australia and D. Wells for assisting with assessing one of the trials in Victoria.

Staff at Medina Research Station, especially Gavin D'adhemar, are thanked for managing the experiments and assisting with harvest and grading.

We thank the Chemistry Centre (WA) for carrying out metalaxyl analyses on soil samples.

We thank carrot growers for allowing access to their properties, especially L. Gazzola and G. Parente for hosting the farm trials.

Funding from AUSVEG/Horticulture Australia Ltd. is gratefully acknowledged.

References

Ali-Shtayeh, M. S. (1985). Value of oogonial and oospore dimensions in *Pythium* species differentiation. *Transactions of the British mycological Society* **85,** 761-764.

Armstrong, J.S., Gibbs, A.J., Peakall, R. and Weiller, G. (1994). 'The RAPDistance Package' ftp://life.anu.edu.au/pub/software/RAPDistance.

Bailey, A. M. and Coffey, M. D. (1985). Biodegradation of metalaxyl in avocado soils. *Phytopathology* **75,** 135-137.

Barr, D. J. S., Warwick, S. I. and Desaulniers, N. L. (1997). Isozyme variation, morphology, and growth response to temperature in *Pythium iregulare*. *Canadian Journal of Botany* **75,** 2073-2081.

Benard, D., and Punja, Z. K. (1995). Role of *Pythium* species in cavity spot development on carrots in British Columbia. *Canadian Journal of Plant Pathology* **17**, 31-45.

Biesbrock, J. A. and Hendrix, F. F. (1967). A taxonomic study of *Pythium irregulare* and related species. *Mycologia* **59,** 943-952.

Breton, D. and Rouxel, F. (1993). Donnees recentes sur le cavity-spot de la carotte en France. *Acta Horticulturae* **354**, 159-170.

Calverly, D. J. and Unwin, J. (1981). Determination of residues of furalaxyl and metalaxyl in nutrient solution, peat compost and soil samples by gas chromatography. *Analyst* **106**, 389-393.

Cohen, Y. and Coffey, M. D. (1986). Systemic fungicides and the control of Oomycetes. *Annual Review of Phytopathology* **24,** 311-338.

Chase, C. A., Sinclair, T. R., Chellemi, D. O., Olsen, S. M., Gilreath, J. P. and Locascio, S. J. (1999). Heat-retentive films for increasing soil temperatures during solarization in a humid, cloudy environment. *HortScience* **34,** 1085-1089.

Davison, E. M. and McKay, A. G. (1998). *Pythium* spp. associated with cavity spot of carrots in Western Australia. *Australasian Plant Pathology* **27,** 163-168.

Davison, E. M. and McKay, A. G. (1999). Reduced persistence of metalaxyl in soil associated with its failure to control cavity spot of carrots. *Plant Pathology* **48,** 830-835.

Dewan, M. .M. and Sivasithamparam, K. (1989). *Pythium* spp. in roots of wheat and rye grass in Western Australia and their effect on root rot caused by *Gaeumannomyces graminis* var. *tritici*. *Soil Biology and Biochemistry* **20**, 801-808.

Dice,L.R. (1945). Measures of the amount of ecologic association between species. *Ecology* **26,** 297-302.

Dick, M. W. (1990). *Keys to Pythium*. MW Dick, University of Reading, UK.

Droby, S. and Coffey, M. D. (1991). Biodegradation process and the nature of metabolism of metalaxyl in soil. *Annals of applied Biology* **118**, 543-553.

El-Tarabily, K., A., Hardy G. E. St. J. and Sivasithamparam, K. (1996). Association of *Pythium coloratum* and *Pythium sulcatum* with cavity spot disease of carrots in Western Australia. *Plant Pathology* **45,** 727-735.

Francis, D. M. and St. Clair, D. A. (1997). Population genetics of *Pythium ultimum*. *Phytopathology* **87,** 454-461.

Galati, A. and McKay, A. G. (1996). Carrot Yield Decline. Final Report, Horticultural Research and Development Corporation Project VG036.

GENSTAT 5 Committee of the Statistics Department Rothamsted Experimental Station, 1993. *Genstat 5 Release 3 Reference Manual.* Oxford, UK: Oxford Scientific Publications.

Guerin, L., Briard, M. and Rouxel, F. (1994). Biochemical characterisation of *Pythium* spp. involved in cavity spot of carrots in France. *Annals of applied Biology* 125, 255-265.

Harman, G. E. (2000). Myths and dogmas of biocontrol. *Plant Disease* **84,** 377-393.

Hendrix and Campbell, W. A. (1974). Taxonomic value of reproductive sell size in the genus *Pythium. Mucologia* **66,** 681-684.

Kageyama, K., Tachi, M., Umetsu, M and Hyakumachi, M. (1996). Epidemiology of *Pythium sulcatum* associated with brown-blotted root rot of carrots. *Annals of the Phytopathological Society of Japan* **62,** 130-133.

Kalu, N. N., Sutton, J. C. and Vaartaja, (1976). *Pythium* spp. associated with root dieback of carrot in Ontario. *Canadian Journal of Plant Science* **56,** 555-561.

Katan, J. (1980). Soil pasteurization of soils for disease control: status and prospects. *Plant Disease* **64,** 450-454.

Kookana, R. S., Di, H. J. and Aylmore, L. A. G. (1995). A field study of leaching and degradation of nine pesticides in a sandy soil. *Australian Journal of Soil Research* **33,** 1019-1030.

Liddell, C. M., Davis, R. M., Nuñez, J. J. and Guerard, J. P. (1989). Association of *Pythium* spp. with carrot root dieback in the San Joaquin Valley of California. *Plant Disease* **73,** 246-249.

Lyshol, A. J., Semb, L., and Taksdal, G. (1984). Reduction of cavity spot and root dieback in carrots by fungicide application. *Plant Pathology* **33,** 193-198.

Matheron, M. E. and Porchas, M. (1999). Development of stem lesions on pepper plants inoculated with *Phytophthora capsici* after treatment with acibenzolar-S-methyl. *Phytopathology* **89,** S49.

Matheron, M. E. and Porchas, M. (2000). Impact or azoxystrobin, dimethomorph, fluazinam, fosetyl-Al, and metalaxyl on growth, sporulation, and zoospore cyst germination of three *Phytophthora* spp. *Plant Disease* **84,** 454-458.

McKay, A. G. and Davison, E. M. (2000). Carrot Export Development. Final Report, Horticultural Research and Development Corporation Project VG 95010.

Montfort, F. and Rouxel, F. (1988). La maladie de la 'tache' de la carotte due à *Pythium violae* Chester et Hickman: données symptomatologiques et étiologiques. *Agronomie* **8,** 701-706.

Nagai, Y, Fukami, M. Murata, A. and Watanabe, T. (1986). Brown-blotted root rot of carrots in Japan. (1) Occurrence, symptoms and isolation. *Annals of the Phytopathology of Society Japan* **52,** 278-286.

Nei, M. and Li, W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Acadamy of Science USA* **76,** 5269-5273.

Pinel, M. C. P., Bond, W. and White, J. G. (2000). Control of soil-borne pathogens and weeds in leaf salad monoculture by use of a self-propelled soil-steaming machine. *Acta Horticulturae* **532,** 125-130.

Plaats-Niterink, A. J. van der (1981). Monograph of the genus *Pythium*. Studies in Mycology No. 21. Centraalbureau voor Schimmelcultures, Baarn.

Powelson, M. L. and Inglis, D. A. (1999). Foliar fungicides as protective seed piece treatments for management of late blight of potatoes. *Plant Disease* **83,** 265-268.

Pratt, R. G. and Mitchell, J. E. (1973). A new species of *Pythium* from Wisconsin and Florida isolated from carrots. *Canadian Journal of Botany* **51,** 333-339.

Raeder, U. and Broda, P. (1985). Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* **1**: 17-20.

Sawar, M., Kirkegaard, J. A., Wong, P. T. W. and Desmarchelier, J. M. (1998). Biofumigation potential of brassicas. *Plant and Soil* **201,** 103-112.

Schrandt, J. K., Davis, R. M. and Nuñez, J. J. (1994). Host range and influence of nutrition, temperature, and pH on growth of *Pythium violae* from carrot. Plant Disease **78,** 335-338.

Shahzad, S., Coe, R. and Dick, M. W. (1992). Biometry of oospores and oogonia of *Pythium* (Oomycetes): the independent taxonomic value of calculating ratios. *Botanical Journal of the Linnean Society* **108,** 143-165.

Shetty, K. G., Subbarao, K. V., Huisman, O. C. and Hubbard, J. C. (2000). Mechanism of broccoli-mediated verticillium wilt reduction in cauliflower. *Phytopathology* **90,** 305-310.

Stapleton, J. J. (2000). Soil solarization in various agricultural production systems. *Crop Protection* **19,** 837-841.

Stirling, A. M., Stirling, G. R. and Macrae, I. C. (1992). Microbial degradation of fenamiphos after repeated application to a tomato-growing soil. *Nematologica* **38,** 245-254.

Sweet, J. B., Beale, R. E. and Wright, I. R. (1989). Cavity spot disease in six carrot cultivars treated with metalaxyl and thiram fungicide. *Annals of applied Biology Supplement* **114,** 38-39.

Tanaka, S., Kochi, S., Kunita, H., Ito, S. and Kameya-Iwaki, M. (1999). Biological mode of action of the fungicide, flusulfamide, against *Plasmodiophora brassicae* (clubroot). *European Journal of Plant Pathology* **105,** 577-584.

Vivoda, E., Davis, R. M., Nuñez, J. J. and Guerard, J. P. (1991). Factors affecting the development of cavity spot of carrots. *Plant Disease* **75,** 519-522.

Vogelstein, B. and Gillespie, D. (1979). Preparative and analytical purification of DNA from agarose. *Proceedings of the National Academy of Sciences of the United States of America* **76**: 615-619.

Walker, A. (1993). Degradation, biodegradation and enhanced biodegradation of pesticides in soil. In: Del Re A. A. M., Capri, E., Evans, S. P. and Trevisan, M. eds., *Proceedings of the Ninth Symposium on Pesticide Chemistry, 1993*. Piacenza, Italy: IUPAC, 357-370.

Walker, G. E. (1988). Control of carrot cavity spot with metalaxyl and phosphorus acid. *Australasian Plant Pathology* **17,** 41-44.

Walker, G. E. (1991). Effect of metalaxyl and phosphonate on incidence of cavity

spot in carrots. *Australasian Plant Pathology* **20,** 21-26.
Wang, P. H. and White, J. G. (1997). Molecular characterization of *Pythium* species based on RFLP analysis of the internal transcribed spacer region of ribosomal DNA. *Physiological and Molecular Plant Pathology* **51,** 129-143.
Warton, B. and Matthiessen, J. N. (2000). Enhanced biodegradation of metham sodium. In: Carrot Association for Research and Development (WA inc.), *Proceedings of Carrot Conference Australia.* Perth, Australia, 36-37.
Watanabe, T., Nagai, Y. and Fukami, M. (1986). Brown-blotted root rot of carrots in Japan (2) Culture and identification. *Annals of the Phytopathological Society of Japan* 52, 287-291.
Wheatley, G. A., Hardman, J. A., and Edmonds, G. H. (1984). Effects of metalaxyl and carbofuran in a fluid-drilling gel on cavity spot incidence and the yield of carrots. *Annals of applied Biology Supplement* **104**, 38-39.
White, J. G. (1986). The association of *Pythium* spp. with cavity spot and root dieback of carrots. *Annals of applied Biology* **108**, 265-273.
White, J.G. (1988). Studies on the biology and control of cavity spot of carrots. *Annals of applied Biology* **113**, 259-268.
White, J. G., Stanghellini, M. E. and Ayoubi, L. M. (1988). Variation in the sensitivity to metalaxyl of *Pythium* spp. isolated from carrot and other sources. *Annals of applied Biology* **113**, 269-277.
White, J. G., Wakenham, A. J. and Shlevin, E. (1993). *Pythium violae* isolated from cavity spot lesions on carrots in Israel. *Phytoparasitica* **21**, 239-243.
Wong, F. P. and Wilcox, W. F. (2000). Distribution of baseline sensitivities to azoxystrobin among isolates of *Plasmopara viticola. Plant Disease* **84,** 275-281.