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.

All expressions of opinion are not to be regarded as expressing the opinion of Horticulture Australia Ltd or any authority of the Australian Government.

The Company and the Australian Government accept no responsibility for any of the opinions or the accuracy of the information contained in this report and readers should rely upon their own enquiries in making decisions concerning their own interests.

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

© Copyright 2001
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.

Any recommendations contained in this publication do not necessarily represent current HA or Department of Agriculture policy. No person should act on the basis of the contents of this publication, whether as to matters of fact or opinion or other content, without first obtaining specific, independent professional advice in respect of the matters set out in this publication.
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Industry summary</strong></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><strong>Technical summary</strong></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><strong>Technology transfer</strong></td>
<td>7</td>
</tr>
<tr>
<td>Section 1</td>
<td>Identification of <em>Pythium</em> spp. from carrots throughout Australia.</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1.1 Identification of <em>Pythium</em> spp. from carrots throughout Australia.</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1.2 Molecular characterisation of <em>Pythium sulcatum</em> isolates from carrots.</td>
<td>24</td>
</tr>
<tr>
<td>Section 2</td>
<td>Victorian and South Australian carrot surveys.</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>R. Brett, R. Cole and E. M. Davison</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.1 Survey of carrot crops in Victoria and south Australia for cavity spot disease.</td>
<td>34</td>
</tr>
<tr>
<td>Section 3</td>
<td>Cultural methods for controlling <em>Pythium</em> diseases of carrots.</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>E. M. Davison, P. A. Murphy and A. G. McKay</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.1 Host range of <em>Pythium sulcatum</em>.</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>3.2 The use of rotation for controlling <em>Pythium</em> diseases of carrots.</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>3.3 The potential for controlling cavity spot disease by soil solarisation.</td>
<td>54</td>
</tr>
<tr>
<td>Section 4</td>
<td>Chemical control</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>E. M. Davison, A. G. McKay and P. A. Murphy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.1 Chemical and microbial control of cavity spot.</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>4.2 Survey of the incidence of enhanced breakdown of metalaxyl on carrot farms.</td>
<td>67</td>
</tr>
<tr>
<td>Section 5</td>
<td>Carrot variety screening for cavity spot tolerance</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>A. G. McKay, E. M. Davison and R. Brett</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.1 Carrot variety screening for cavity spot tolerance 1999 to 2001 plantings</td>
<td>69</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td></td>
<td>79</td>
</tr>
<tr>
<td>References</td>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>
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 °C. It is unlikely to control *P. sulcatum* however, because it survived for 2 hr. at 45°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°C while P. sulcatum survived for 2 hr. at 45°C, and 6 hr. at 42.5°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.


- 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.


- 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 IRI, 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.


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


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
Discussion

The two *Pythium* spp. that are the most important cause of cavity spot worldwide 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.](image-url)
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 areas 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.
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 morphometrics?

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 apertotic 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 apertotic, and number and position of the antheridia. In a study of *P. irregulare*, Biesbrook 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, R. Cole 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.
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.
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. frutescens*), 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 \textit{P. sulcatum}, it is important to confirm that these are non-hosts.

In this experiment we have firstly determined whether \textit{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 \textit{P. sulcatum}. These vegetables include some of those reported as hosts by Kalu \textit{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 \textit{P. sulcatum} used in the experiment.

<table>
<thead>
<tr>
<th>Host</th>
<th>Common name</th>
<th>Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Daucus sativa}</td>
<td>Carrot</td>
<td>Ivor</td>
</tr>
<tr>
<td>\textit{Cucumis melo}</td>
<td>Musk melon</td>
<td>Hales Best</td>
</tr>
<tr>
<td>\textit{C. sativus}</td>
<td>Cucumber</td>
<td>Burpless</td>
</tr>
<tr>
<td>\textit{Phaseolus vulgaris}</td>
<td>Bean</td>
<td>Brown Beauty</td>
</tr>
<tr>
<td>\textit{Avena sativa}</td>
<td>Oats</td>
<td>Mortlock</td>
</tr>
<tr>
<td>\textit{Hordeum vulgare}</td>
<td>Barley</td>
<td>Mighty Mouse</td>
</tr>
<tr>
<td>\textit{Secale cereale}</td>
<td>Rye</td>
<td></td>
</tr>
<tr>
<td>\textit{Triticum aestivum}</td>
<td>Wheat</td>
<td>Eradu</td>
</tr>
<tr>
<td>\textit{Zea mais}</td>
<td>Maize</td>
<td>Honesweet</td>
</tr>
</tbody>
</table>

**Seedling harvest**

The experiment was harvested on 27.2.01. Twenty seedlings were carefully removed from each plot, and stored overnight at 4°C 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 \textit{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°C. \textit{Pythium} spp. were identified on their colony and hyphal morphology; \textit{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.

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

* = 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°C. 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
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-methylthiobutanyl- 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°C while *P. sulcatum* survived for 2 hr. at 45°C, and 6 hr. at 42.5°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°C at 10 cm and 43/37°C at 20 cm with a 35/20°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°C at 10 cm and 36/25°C at 20 cm with a 40/15°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 (Plaat-Niterink, 1981) at 20°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 31. Survival of *P. violae* isolates at different time x temperature combinations: +: growth within 12 days, -: no growth within 12 days.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>6</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>32.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>35.0</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>40.0</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*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 (00mdl15)

Summary. A range of chemical (acibenzolar-S-methyl, azoxystrobin, didecyldimethylammonium 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 enhanced 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.
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°C and sub-samples taken after 2 and 4 weeks. All sub-samples were stored at −20°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:

$$\text{half-life} = -(1/C)\cdot \ln((T_0/2-A)/B)$$

where \(A\), \(B\) and \(R\) are values from the fitted curve \(A+B\cdot R^X\),
\(C = (-\ln(R))\),
and \(T_0 = (A+B)\).

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>
<thead>
<tr>
<th>State</th>
<th>Post code</th>
<th>Number of samples</th>
<th>Half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>5355</td>
<td>2</td>
<td>28-29</td>
</tr>
<tr>
<td>SA</td>
<td>5341</td>
<td>5</td>
<td>21-23</td>
</tr>
<tr>
<td>TAS</td>
<td>7306</td>
<td>4</td>
<td>nd</td>
</tr>
<tr>
<td>TAS</td>
<td>7310</td>
<td>20</td>
<td>6-24</td>
</tr>
<tr>
<td>WA</td>
<td>6065</td>
<td>4</td>
<td>28-30</td>
</tr>
<tr>
<td>WA</td>
<td>6167</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>WA</td>
<td>6171</td>
<td>8</td>
<td>&lt;1-43</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>44</td>
<td>&lt;1-43</td>
</tr>
</tbody>
</table>

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

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

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

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.