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Managing alternaria blight in carrots

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SA Research &
Development Institute

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Managing Alternaria Blight in Carrots



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HAL Project VG00014

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This report is the result of 3 years investigations into Alternaria blight, caused by *Alternaria radicina*. This fungus was found to be the cause of poor seedling establishment in the carrot industry.

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November 2003

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Front page: Application of controls (water only) and fungicides to six-week carrot seedlings using a watering can. Plots 10m in length, four row of seedling/raised bed, Virginia 2001

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MEDIA SUMMARY

Poor establishment of carrot seedlings was associated with high levels of infection by the fungus *Alternaria radicina*. Seedling losses were wide spread in all growing regions in SA. On some properties seedling losses were up to 47%. *A. radicina* is seed-borne and tests of commercial seed from Australia and overseas showed that 18 of the 19 lines of seed were infected, with the highest infection level of 35% found for imported seed. Many of these findings have been outlined in a publication for the journal Australasian Plant Pathology (2003) **32**: 99-104 (Appendix 2)

Fungicide drenches of Amistar and Rovral on 6 to 8 week seedlings controlled infections by *A. radicina*. Results of field trials and residue studies were submitted to the CPA to support registration for minor use of these fungicides on carrots. Field trials have demonstrated that fungicides applied to carrot umbels before infection reduced the seed-borne levels of *A. radicina*. Seed treatments were evaluated as a means of reducing or eliminating *A. radicina* from commercial seed. Complete control was achieved with steam treatments at 51°C for 30 mins or hot water at 50°C for 30 mins. Potassium permanganate soaks and hydrogen peroxide baths also significantly reduced seed-borne infestations.

Micro-organisms isolated from carrot seed and soils were found to have potential as seed applications of biological antagonist of *A. radicina*. Up to 99% control of seed-borne *A. radicina* infestation was achieved using a 10 min seed soak of a fungal antagonist. Similar findings have been reported in Denmark, for the production of safe organic carrots. Further research on imported and locally produced seed is required.

Green house trials to compare carrot cultivars for resistance to *A. radicina* demonstrated that smaller varieties often grown for specialty fresh markets are more resistant than common commercial varieties.

TECHNICAL SUMMARY

Alternaria radicina was shown to be widespread throughout SA and introduced into growing areas by the use of infested seed. The fungus attacks all stages of carrots in the field, causing seedling damping-off, rotting of roots, crowns and petioles of seedlings and leaves of mature carrots. Soil tests have shown that levels of up to 347 colony forming units (CFUs)/g of soil occur in areas with a history of carrot production. Overseas research has shown some levels above 20 CFUs can cause an outbreak of *A. radicina*. Soil treatments with Metham and Telone reduced *A. radicina* to 1 CFU/g at planting. By harvest the levels had risen to 35 CFU/g for Metham, 47 CFU/g for Telone, while untreated sites were lower at 29 CFU/g. The result suggests that soil treatments also reduce *A. radicina* antagonistic organisms such as *Streptomyces* spp. Other management strategies to reduce soil levels of *A. radicina* include rotation with non-host crops such as barley, broccoli, beetroot, Tricali and other Brassica crops. Soil tests have show that these rotations reduce the level of *A. radicina* CFUs/g of soil.

Fungicide drenches (fungigation) to 6 and 8 week seedlings demonstrated that Amistar (azoxystrobin) was the most effective fungicide to control crown and petiole infection by *Alternaria*. Rovral Aquaflow (iprodione) was less effective but better than Score (difenoconazole) or Sumislex 500 (procymidone). Rovral also reduced the number of *Alternaria* colonies in the soil up to 4 months after fungigation.

Fungicide trials on artificially infected seed crops showed BAS 510 (Filan), BAS 500 (Cabrio) and Amistar applied 7 days before infection were better than Sumislex 500 and Score for the control of *A. radicina*.

Base line sensitivity studies were carried out with the fungicides Rovral, Thiram, and Amistar using a variety of *Alternaria* isolates from seed, seedlings and soil. One *A. radicina* isolate from fungicide-coated seed grew in the presence of iprodione at 50 and 500mg/L whereas sensitivity was high in other isolates.

A variety of seed treatments were evaluated as means of controlling seed-borne *A. radicina* infestation. Complete control was achieved with steam treatments at 51°C for 30 mins or hot water at 50°C for 30 mins. Potassium permanganate soaks and hydrogen peroxide baths also significantly reduced seed-borne infestations. Promising control of *A. radicina* infested seed was achieved using antagonists *Gliocladium* spp and a *Penicillium* spp. Several *Streptomyces* spp isolated from soil were also parasitic to *A. radicina*.

Cultivar resistance trials show that smaller varieties often grown for specialty fresh markets such as All Seasons, Amsterdam, Golfball, Imperator and tropical varieties of Kuroda are more resistant to *A. radicina* than the more common commercial varieties, i.e. Danvers and Nantes types that include: Carrisma, Stephano and Havana.

INTRODUCTION AND REVIEW OF LITERATURE

The research findings in this report follow on from the HRDC project “Investigative study into carrot seedling establishment and disease management, Feb 2000”. This report outlines the results of the extended survey undertaken to determine the extent of the problem in SA and investigations to formulate management strategies.

Carrot growers in SA have reported losses due to poor seedling establishment and damping-off since the 1970s. Damping-off occurs unpredictably, usually during periods of warm humid weather, and has not been successfully controlled by fungicide applications such as thiram and iprodione. Work in Queensland has established that *Rhizoctonia* and *Pythium* were shown to cause damping-off of carrot seedlings in Queensland (R. G O'Brien unpublished data) and Tasmania (H.Pung pers. com). In Western Australia, *Pythium* spp. were pathogenic to carrot seedlings causing damping-off and cavity spot in mature carrots (Davison and McKay, 1998). A limited survey in the Virginia area in 1994 implicated *Alternaria* and *Fusarium* as possible causes of carrot seedling damping-off (T. Wicks, unpublished data), but no pathogenicity tests were carried out to confirm this.

The introduction of carrot fungal pathogens into the soil in growing areas has been attributed to the use of infested seed. The most serious seed-borne fungal pathogens are *Alternaria radicina*, *A. radicina* var. *carotiincultae* (Davis and Raid, 2002) and *A. dauci* (Pryor et al, 2002).

A. radicina has been recorded world wide from celery, celeriac, caraway, dill, fennel, parsley and parsnip (Davis and Raid, 2002). Wearing (1980) recorded *A. radicina* at Angle Vale on Celery in 1977-78 as the first record in SA and demonstrated re-infectivity on carrots. In a recent survey in South Australia, *A. radicina* was regularly found in infested seed and soil and isolated from all parts of carrots (HRDC report Feb 2000, VG 98000). *A. radicina* causes black lesions on the petioles, which advance to the stem, hypocotyl and upper root surfaces. It also causes seedling damping-off, harvest and cold storage losses (Pryor et al 1998). Long-lived spores survive in crop debris and soil.

Fungicide applications to control foliar infection of carrots by *Alternaria* spp. were investigated by Langenberg (1975). Gillespie and Sutton (1979) provided a predictive scheme for timing fungicide applications to control *Alternaria* leaf blight in carrots using protectant fungicide Bravo (chlorothalonil) and the systemic fungicide Bayleton (triadimefon). Galati and McKay (1995) found that under high disease pressure, alternating weekly Bravo and Dithane (mancozeb) gave excellent blight control. Kocide (copper hydroxide) could also be alternated with chlorothalonil. It was recommended that sprays be applied at the first sign of disease. Rovral (iprodione) and Sumisclex (procymidone) failed to control leaf blight in Western Australian trial work (Galati and McKay, 1995). Two foliar applications of gibberellic acid GA at 40mg/L or less reduced the severity of *Alternaria* leaf without affecting root quality, and was similar to that achieved by four applications of the fungicide iprodione (Rovral), (Santos et al., 2003). In Israel, Ben-Noon et al (2001) demonstrated *Alternaria* leaf blight was more effectively controlled by the systemic fungicides Score (difenoconazole) than the translaminar fungicide Rovral (iprodione). Also, the irrigation practice of drip irrigation, instead of overhead sprinklers, with the use of fungicide sprays, greatly reduced foliar infection by *Alternaria dauci* (Ben-Noon et al., 2003).

In an effort to reduce seed-borne disease levels, Shimmer (1953) recommended a hot water seed soak at 50°C for 18 min. This method had the disadvantage of reducing seed viability and did not completely eradicate disease. Maude (1966 and 1969) recommended a method of soaking seed in a 0.2% aqueous suspension of Thiram (thiram) for 24 hours at 30°C that eliminated *Alternaria* from the carrot seed. An improvement in application of fungicides to seed was to use organic solvents such as acetone or dichloromethane (Kar-Ling Tao et al, 1974). These methods were more effective than dusting or coating the seed with thiram, however it was best suited for small batches of seed (Harris, 1975).

In a 1977 South Australian Department of Agriculture fact sheet, a cheap method was recommended for seed-borne disease control. It consisted of hot water soaks at 50°C for 25 to 30 mins. For large-scale seed treatments machines producing steam/air mixtures are more effective but often too expensive for the individual growers to use (Wicks, 1977). Soteris (1979) and Pryor et al (1994) tested carrot seed treatments using sodium hypochlorite solutions (0.1 to 1%) and thiram (0.2%) and iprodione (0.2%) at 100°C for 1 hour and 50°C for 10min to 4 hours. It was found that hot water (50°C for 30 min) or hot sodium hypochlorite (0.1% or 1.0%) at 50°C for 30 min eradicated *A. radicina* from infested carrot seed with a minimal reduction in seed germination (Pryor et al, 1994). While hot fungicide soaks of 24 hours (0.2% a.i. iprodione or thiram) at 30°C have been reported to successfully eradicate *A. radicina* from seed, this practice is not used commercially and it was found that shorter treatment times of fungicide soaks at 30°C were not sufficient to eradicate *A. radicina* (Pryor et al, 1994). Generally seed companies dust carrot seed with thiram at a rate of 5g/kg of seed, however dusting is not as effective as soaking (Galati and McKay, 1995). A procedure that can be integrated into a management strategy for *Alternaria* is a relatively simple PCR-based assay for the detection of seed-borne *A. radicina*. This method detects infestation rates as low as 0.3% (Pryor and Gilbertson, 2001).

With the move towards organic farming in the late 1990s, a resurgence in the need for carrot seed free of disease and fungicides has occurred. This has stimulated the re-use hydrogen peroxide seed treatments (Normand and Fortin, 1982), hot water treatments and seed treatments with bio-control fungi such as *Trichoderma harzianum* and *Streptomyces griseovirides* (Hermansen et al, 1999). No effect was found to occur with carrot yield and storage quality, but a reduction in the incidence of the saprophyte *Ulocladium atrum* on the seeds was observed. Hermansen et al (1999) also found that hot water treatments alone at 54°C for 20 min eradicated *A. dauci* without adversely affecting germination, however these findings may not be significant because of the low number of seeds tested i.e. 300-400 does not represent a meaningful sample.

Seed batches in the USA, which had been soaked in fungicide, still can have an infestation rate of 0.1% (Pryor et al, 1994).

Knudsen (2003) investigated the biological control of seed-borne *Alternaria* spp to enable the production of safe organic carrots. Biopriming of carrot seed was achieved by soaking in a conidial suspensions of 10⁴ and 10⁵ spores/ml of *Clonostachys/Gliocladium* spp. Suppression of *A. radicina* and other *Alternaria* spp was achieved for naturally infected carrot seeds. Additional suppression of *A. radicina* on seeds artificially inoculated with *A. radicina* was achieved by coating with a peat-bran formulation of selected antagonists. *Clonostachys/Gliocladium* spp. improved carrot seedling emergence at the same level of iprodione treatment.

TECHNICAL REPORT

FUNGAL PATHOGENS RECOVERED FROM CARROTS

Materials and methods

Between 1999 and 2003, isolations were made from diseased carrots collected from various commercial plantings in South Australia and Victoria

Methods used in this survey of carrot crops, isolation of fungi from plants and soil and testing for pathogenicity have been described in a recent publication (Coles and Wicks, 2003) shown in Appendix 2.

Results and discussion

Eighteen different fungi were recovered from carrots at various locations (Table 1).

Table 1. Fungi recovered from carrots

Name of pathogen	Locations and source
<i>Alternaria radicina</i> *	All SA carrot growing areas, all carrot stages
<i>Alternaria radicina</i> var. <i>carotiincultae</i> *	Virginia, Paringa, and Kybybolite, seed, reject and cold storage carrots
<i>Alternaria dauci</i> *	Paringa and Parilla, on leaves and imported seed
<i>Cercospora carotae</i> *	Virginia, Nuriootpa and Mount Gambier, leaves and seed crops
<i>Fusarium solani</i> *	Virginia, Blanchetown and Paringa, seedlings and harvested carrots
<i>Pythium sulcatum</i> *	Virginia, Paringa, Nuriootpa, Mt Gambier, seedlings and reject carrots
<i>Pythium violae</i> *	Paringa, reject carrots
<i>Botrytis cinerea</i> *	Virginia and Kybybolite, reject carrots and seed crops
<i>Sclerotinia rolfsi</i> *	Virginia and Blanchetown, pre-harvest carrots
<i>Thielaviopsis basicola</i> *	Kybybolite, cold storage carrots
<i>Alternaria arborescens</i>	Kybybolite, carrot umbels
<i>Alternaria tenuissima</i>	Imported seed
<i>Alternaria alternata</i>	All SA carrot growing areas, all carrot stages
<i>Fusarium equiseti</i>	Kybybolite and Virginia, seedling roots
<i>Streptomyces</i> spp	Waikerie, Virginia and Victoria, reject carrots
<i>Pleospora herbarum</i>	Kybybolite and Mt Gambier, on leaves and imported seed.
<i>Stemphylium botryosum</i>	All SA carrot growing areas, leaves and seed
<i>Gliocladium auream</i>	Virginia, carrot necks and imported seed

* Indicates fungi that are pathogens of carrots

A. radicina, *A. carotiincultae*, *A. dauci* and *Cercospora carotae* are considered to be specific pathogens of carrots and some other Umbelliferae spp (Davis and Raid, 2002). Symptoms of the *Alternaria* spp infection include seeding damping-off, and rotting of roots, crowns, seedlings, petioles, leaves and crowns of mature carrots (Coles and Wicks, 2003). Initially *C. carotae* causes lesions on leaves similar to those caused by *Alternaria* spp. They appear as necrotic flecks and soon develop a chlorotic halo. As the lesions grow they coalesce causing leaflets to wither, curl and die. *C. carotae* usually precedes *Alternaria* leaf blight but does not affect the carrot tap roots. *C. carotae*, *A. radicina* and *A. dauci* are seed-borne and are introduced into soil by the use of infested seed.

Both *Pythium sulcatum* and *P. violae* were infrequently recorded on carrots in SA (Davison and McKay, 2001). *P. sulcatum* occurs on other vegetables and *P. violae* also occurs on cereal. *P. sulcatum* is the commonest cause of cavity spot in carrots and is found in most production areas of all states. *P. violae* also causes this disease in irrigated properties along the River Murray. Both are slow growing pathogens of carrots that also cause, damping-off and root dieback, resulting in forked or misshapen carrots (Davison and McKay, 2003).

F. solani was frequently recorded on harvest carrots during autumn and spring in most growing areas. The disease is referred to as 'Dry Rot' and occurs more frequently on carrots held in the field after maturity. The fungus is soil and seed-borne and infection occurs through insect wounds or damage caused by other fungi (Davis and Raid, 2002).

B. cinerea, *S. rolfsi* and *T. basicola* were occasionally isolated from pre-harvest carrots, tap roots of seed crop plants and colds storage carrots. These fungi are saprophytic and have a large host range. They were common in soils where other susceptible crops were used in rotation. Their incidence was reduced when small grain crops were used in rotations (Davis and Raid, 2003).

The remainder of the fungi listed in Table 1 are either weak pathogens or saprophytes of carrots (Davis and Raid, 2003)

LEVELS OF *A. RADICINA* IN SOILS IN SA

Soils from different growing regions were collected during 2000 - 2003. Levels of *A. radicina* in the soil were measured to determine the populations in various soils and the survival of *A. radicina* after different fungicide treatments, fumigation rotation practices

***Alternaria radicina* in carrot soil**

Materials and methods

The level of *A. radicina* in soil was measured using a selective agar (ARSA) (Appendix 1). 25 soil cores were taken to a depth of 15cm in a zigzag pattern. Soil samples were dried for a week at room temperature, crushed and mixed. 4gm of dried soil was added to 40ml of 0.2% water agar and 1ml of the solution spread over the surface of 3 ARSA plates. After 10 days incubation in darkness at 30°C, the number of colonies of *Alternaria* were assessed and recorded as the number of colony forming units (CFU)/g soil. Identifications were made using a 40X magnification binocular microscope.

Results and discussion

All carrot-growing areas in SA had varying levels of *A. radicina* in the soil (Table 2). Six of the eight locations had levels above 20 CFUs/g soil. This measurement and above is capable of causing a disease occurrence in the field (Pryor, 1994). Higher levels were measured in soil that had a history of two years or more of carrot seed production and a 20-year history of carrot production. In areas where soil had been fumigated, the re-establishment of the disease was attributed to the use of infested seed.

Table 2. *Alternaria radicina* levels (CFUs/g) in carrot growing soil, South Australia

Location sample date	Mean CFUs/g of soil	Range CFUs/g of soil	Comments
Paringa 27/10/00	42	0-70	<i>A. radicina</i> damping-off, infected seed
Paringa 27/1/00	0	0	No <i>A. radicina</i> after rape and beetroot rotation
Paringa 27/1/00	0	0	No <i>A. radicina</i> in wheat crop*
Blanchetown 3/5/00	210	190-220	<i>A. radicina</i> infection of crowns 20 year history of carrot growing, no soil fumigation.
Blanchetown* 3/6/00	347	300-410	<i>A. radicina</i> infection of crowns 20 year history of carrot growing, no soil fumigation
Blanchetown*27/9/00	<1	0-4	Wheat crop, soil depths to 25cm*
Parilla 26/1/00	348	180-480	<i>A. radicina</i> damping-off stunted seedlings
Virginia 19/4/00	3	0-30	<i>A. radicina</i> low after soil fumigation and foliar fungicide treatment and fungigation
Kybybolite 1, 15/11/99	14	0-40	Healthy seedling soil
Kybybolite 2 15/11/99	92	80-170	Diseased seedling soil
Waikerie 1 12/10/00	43	20-60	Soil fumigated 6 months previously
Mt Gambier 24/10/00	10	0-20	Carrot seed crop for two years

* See Figure 1.

Alternaria radicina levels in soil at various depths

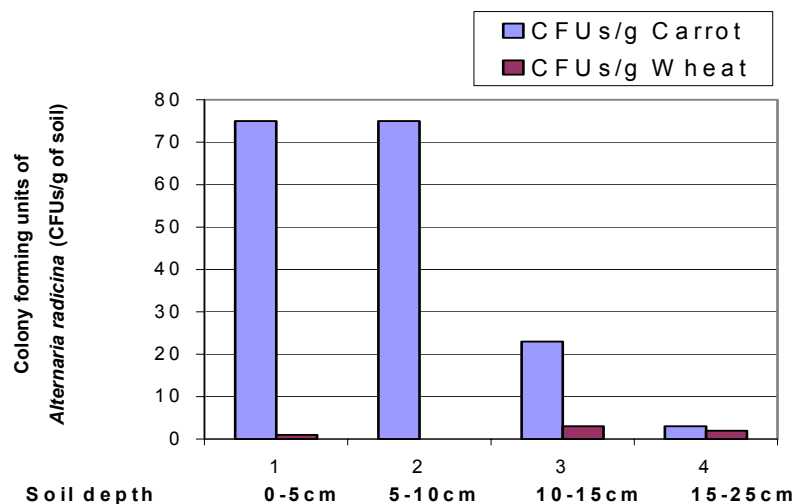
Materials and methods

Levels of *A. radicina* in soils sampled at different depths were measured in a carrot planting near Blanchetown during September 2000. The site had a history of carrot production with *A. radicina* outbreaks occurring over 20 years. In May to June 2000 levels were measured at 210 to 347 CFUs/gm of soil 15cm. After carrot harvest in September 2000 the area was sampled at five depths, 0-5cm, 5-10cm, 10-15cm, 15-20cm and 20-25cm. Soils were collected from four carrot diseased sites and four sites in a wheat crop situated 200m north of the carrot planting, and levels of *A. radicina* assessed as previously described.

Results and discussion

High levels of *A. radicina* were found in the top 10cm layer of soil near diseased carrots and areas with a history of *A. radicina* (Fig 1). The level of *A. radicina* in carrot soil averaged 75 CFUs above 10cm, dropping to CFUs at 15 to 25cm depth. In comparison, in the wheat soil more *A. radicina* was found below 10cm, but at lower levels (1-3CFU/g)

The populations of *A. radicina* would be expected to be higher in the top levels in the soil surrounding the carrot crop. It is likely the fungus was incorporated into the lower 25cm soil profile by the post harvest cultivation of infected carrot tissue. Transfer of infected leaf material or soil probably caused the contamination of soil into the nearby wheat growing area. Continued cultivation of the upper levels in the absence of a host crop



would reduce the fungal population.

Figure 1. Levels of *Alternaria radicina* in soil from different depths in a diseased carrot crop and an adjacent wheat crop, Blanchetown September 2000

***A. radicina* levels in the soil after fungicide drenches**

Materials and methods

The soil levels of *Alternaria* were measured near harvest, four months after a fungigation trial at Virginia (see Experiment 2). 25 soil cores were taken from each plot and levels of *A. radicina* assessed as previously described.

Results and discussion

The soil levels of *Alternaria* in the untreated plots averaged 82 CFUs/g. All fungicides reduced *Alternaria* levels by an average of 63% (Fig 2). In order of efficacy percent reductions were: Rovral 76%, Score 67%, Amistar 55% and Sumisclex 51%. Standard deviations are shown as error bars.

The reduction in the soil-borne levels of *A. radicina* shows that Rovral and other fungicides can influence the pathogens survival in the soil. The application of the fungicides by fungigation into the upper 10cm soil profile reduces the soil population levels of *A. radicina* for up to four months from 82 CFUs/g to 20 CFUs/g of soil.

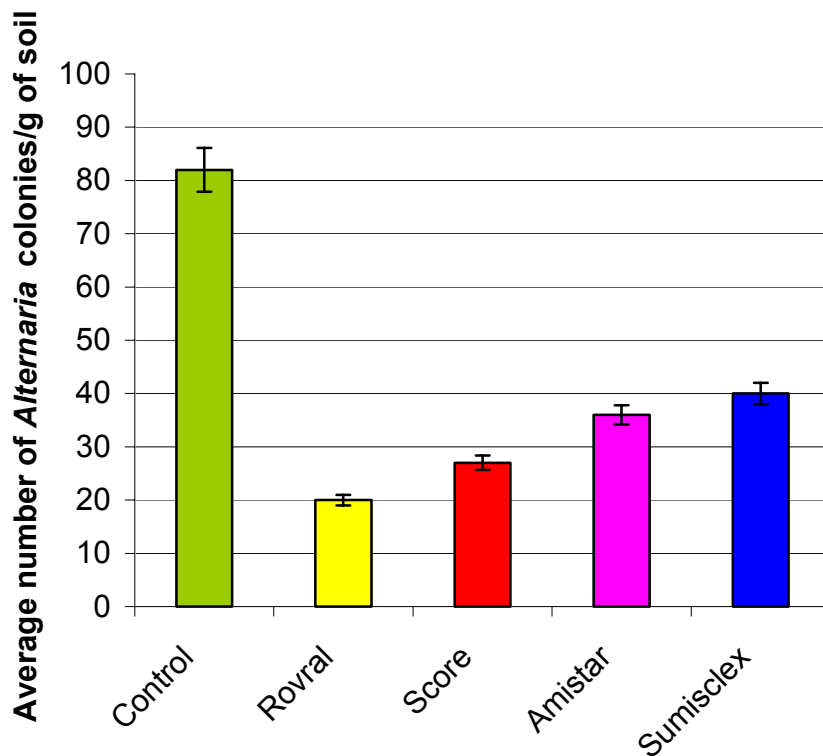


Figure 2. The mean numbers of *Alternaria* colonies in soil 4 months after fungigation on 3/5/01 with fungicides from Experiment 2, Virginia, 2001.

The incidence of *A. radicina* in soil over time after difference management practices

Materials and methods

At Paringa, a large carrot growing area in the Riverland of SA, levels of *A. radicina* in the soil were regularly measured as previously described. Soil was collected between 1999 and 2003 from pivots with a history of different crop management and rotation practices.

Overseas work has shown that there is a significant positive correlation ($P \leq 0.05$) between soil inoculum levels and the incidence of *A. radicina* on carrots in the field. When the population density was greater than 20 CFUs/g soil, the incidence of *A. radicina* in the field ranged from 4 to 91% (Pryor, et al, 1994). The threshold index was calculated as the number of times *A. radicina* levels were over 20 CFUs/g of soil.

Results and discussion

Results show levels of *A. radicina* in the soil remained high in sections of Pivot 18 for one year after planting and fallow (Table 3). A rotation with barley or Brassica crops in old ground reduced the soil levels of *A. radicina*. In sample 18(1), collected from new ground without a history of carrot plantings, levels were 2 CFUs/g, whereas from the same area but in sample 18(2) collected at harvest after 1 crop of carrots, levels were 43 CFUs/g.

Table 3. Levels of *A. radicina* from various cropping sequences at Paringa

Pivot No.	Sample date	Pivot use	CFUs/g of soil	Threshold index *
12	4/10/1999	Carrots, old ground	60	3
8	13/9/2000	Carrots, new ground	0	0
10	13/9/2000	Carrots, new ground	0	0
19	13/9/2000	Carrots, new ground	0	0
11	10/8/2002	Carrots, old ground	150	7.5
11	16/9/2002	Carrots, old ground	150	7.5
11	27/3/2003	Carrots, old ground	50	2.5
18 (1)	27/10/2002	Carrots, new ground	2	0.1
18 (2)	10/5/03	Harvest, fallow	43	2.2
18A	2/6/03	Old ground, barley	7	0.4
18B	27/10/02	Old ground	2	0.1
18B	27/3/03	Carrots,	40	2
18B	10/5/03	Harvest, fallow	13	0.7
17A	10/5/03	Fallow	7	0.4
17C	2/6/03	Old ground, brassica	17	0.9
D overheads	10/5/03	Consecutive carrots	77	3.9
22	10/5/03	New ground, seedlings	0	0
14AD	2/6/03	New ground, harvest	3	0.2
14C	2/6/03	Old ground	17	0.9
23BC	2/6/03	New ground, seedlings	0	0

*The threshold index is the number of times *A. radicina* levels are over 20CFUs, Pryor et al (1994)

These results show that a variety of crop rotations and management practices can reduce the levels of *A. radicina* in the soil. A reduction in *A. radicina* levels occurred after rotations with rape, canola and broccoli. Similar findings of carrot disease reduction in the soil have also been reported for *Pythium* spp (cavity spot of carrots), McKay and Davison (2000).

Carrot growers in SA rotate with *Triticum*, barley, beetroot, broccoli, rape, canola and other Brassica species. These crops are not hosts for *A. radicina*, so soil levels are reduced. Also Brassicas are known to release isothiocyanates into the soil which act as bio-fumigants (Matthiessen et al., 2001). Brassica crop rotations can also be maximised by wet mulching, followed by irrigation allowing ITCs to penetrate into the soil (J. Matthiessen per.com.). High levels of *A. radicina* at Paringa (150 CFUs), Parilla (348 CFUs) and Blanchetown (347 CFUs) were shown to survive in the top 5cm of soil and to a depth of 25cm (Tables 2,3). Crop rotations favourable for disease development were carrots, parsnips and celery, which can be hosts for *A. radicina*. Potatoes were also not suitable as a crop rotation between carrots because the organisms that cause potato common scab, such as *Streptomyces acidiscabies*, *S. caviscabies* and *S. scabies* also affect carrot (Goyer and Beaulieu, 1997).

The effect of soil fumigants on *A. radicina* and *Streptomyces* spp.

A trial (HAL project VG99020) set up to evaluate the effect of fumigants on nematodes in carrots provided an opportunity to measure the levels of *A. radicina* in soils treated with commercial fumigants and to measure levels of *Streptomyces* spp which are known to be antagonists to some fungal root pathogens.

Materials and methods

In four separate plots, seven replicates each of Telone C35 at 520L/Ha and two separate rates of Metham, (1) Low: 300L/Ha and (2) High: 525L/Ha were compared to plots with no treatments.

Soil was sampled before fumigation, and approximately every 4 weeks until harvest. 10 cores were sampled from each plot, as the level of *A. radicina* assessed as previously described.

Results and discussion

Fumigation with Metham and Telone reduced the soil levels of *A. radicina* and *Streptomyces* spp at harvest compared to the control (Fig 3). However in all treatments, the initial pre-fumigation levels of under 1 CFU *A. radicina*/g of soil had increased by harvest to levels above the disease threshold of 20 CFUs, with 29, 35 and 47 CFUs in the control, Metham and Telone treatments respectively.

The higher levels of *A. radicina* in the treated plots compared to the control indicate the presence of possible suppressive organisms that were also being killed by the fumigants. Some *Streptomyces* spp are known to suppress soil-borne plant pathogens in Australian soils (Broadbent et al, 1971). The levels of *Streptomyces* spp were reduced by the application of

Metham and Telone and did not increase to pre-treatment levels by harvest (Fig 4). For example pre-planting *Streptomyces* levels of 319 CFUs were reduced to 145 CFUs after fumigation with Telone, and had only increased to 163 CFUs by harvest. Levels in the control remained stable at over 500 CFUs (Fig 4). The results suggest that early reduction of *Streptomyces* spp by the fumigants caused a reduction in the suppressive activity against *A. radicina*. More work is needed to determine if levels of *Streptomyces* spp and *A. radicina* are related.

While the use of fumigants reduces crown infection, with 23, 9.3 and 7.7% of carrots infected in the control, Metham and Telone treatments respectively, they also increased the levels of *A. radicina* in the soil and the subsequent carry over into the next crop.

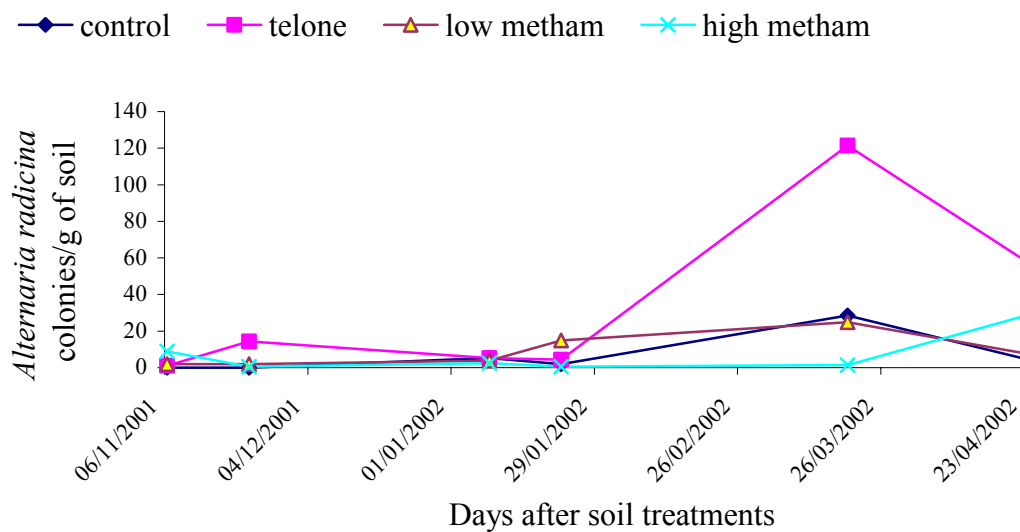


Figure 3. Levels of *A. radicina* in carrot soil after fumigation

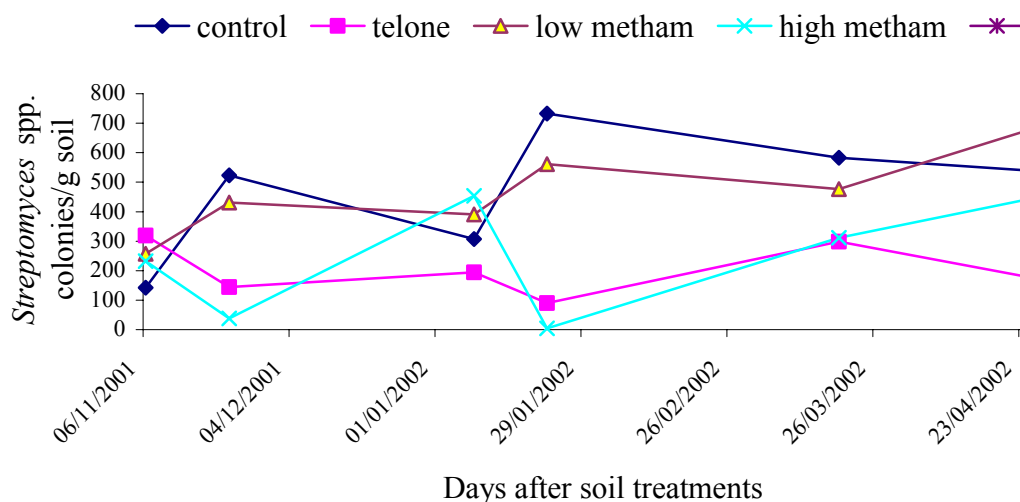


Figure 4. Levels of *Streptomyces* spp in carrot soil after fumigation

FUNGICIDE DRENCHING TRIALS ON CARROT SEEDLINGS

The aim of these trials was to determine the most effective fungicide drench (fungigation) to control *A. radicina* infections on the neck and petioles of carrot seedlings.

Materials and methods

Three trials were set up on grower's properties in Virginia where fertilisers and chemicals are usually applied through irrigation systems. Applications by this method are less damaging to a young crop than boom spraying, less costly and preferred by the industry. To simulate "fungigation", the application of fungicides were applied by a watering can at an irrigation rate of 20,000 litres of water /Ha. The fungicides Amistar, Rovral, Score and Sumislex were applied to six and eight week old seedlings at rates shown in Table 4. Another fungicide Agrifos 400 was also included for comparison in one of the trials as farmers apply the product to control cavity spot of carrots (*Pythium* spp.).

In Experiment 1, treatments were applied to 6-week-old seedlings in April 2001. In Experiment 2 and 3, 8-week-old seedlings were treated in March 2001 and July 2001 respectively.

Fifty carrot seedlings per treatment were collected 14 days after the fungicide applications. For each carrot four replicate sections were taken from the crown and petioles. The presence of *Alternaria* species was determined by surface sterilizing the sections of tissue in 70% ethanol and plating the 200 pieces/treatment on 1/2 PDA (potato dextrose agar). After 10 days incubation at 30°C, tissue pieces were examined under a dissecting microscope at 40X magnification for the presence of *Alternaria* species.

Two weeks before harvest ten carrots were randomly selected from each replicate plot in Experiment 2; washed, dried and leaves removed to the same level on the collar. Total weights of the five replicate treatments were measured.

Table 4. Rates of fungicides used in field and pot trials

Fungicides	Active ingredients	Rates of product /Ha
BAS 510 Filan	500g /Kg boscalid	450g
BAS 500 Cabrio	250 ml/L pyraclostrobin	250ml
Bravo	720g /L chlorothalonil	750g
Amistar	500g /Kg azoxystrobin	450g
Rovral Aquaflo	500g /L iprodione	1L
Scala	400g /L pyrimethanil	1.5
Score	250g /L difenocanazole	1L
Sumislex 500	500g /L procymidone	1L
Agrifos 400	400g /L phosphorus acid	3.5L
Control	-	-

Results and Discussion

Field trials showed that Amistar was consistently the most effective fungicide to control crown and petiole infection by *Alternaria* (Fig 5-7). Results of three trials showed the lowest frequency of isolations were from the Amistar treatments. Rovral was less effective but better than Score or Sumislex. Agrifos, which is mainly applied to control *Pythium*, had no effect on seedling infection by *Alternaria*.

The mean carrot weight was highest in the untreated and Sumislex treatments, and lower weights were recorded in the Rovral, Score and Amistar treatments (Fig. 8). The loss of seedlings through reduced control of *A. radicina* in the untreated and Sumislex treatments would reduce the density of planting and thus allow the carrots to grow larger than where seedlings remained at a higher density.

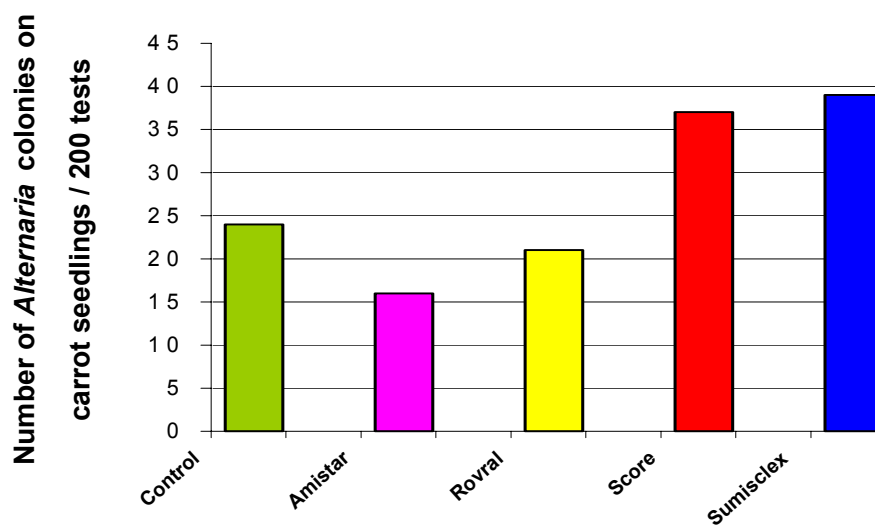


Figure 5. Number of tissue pieces with *Alternaria* on crowns of carrot seedlings treated with various fungicides on 5/4/01 at 6 weeks old. Experiment 1, Virginia 2001

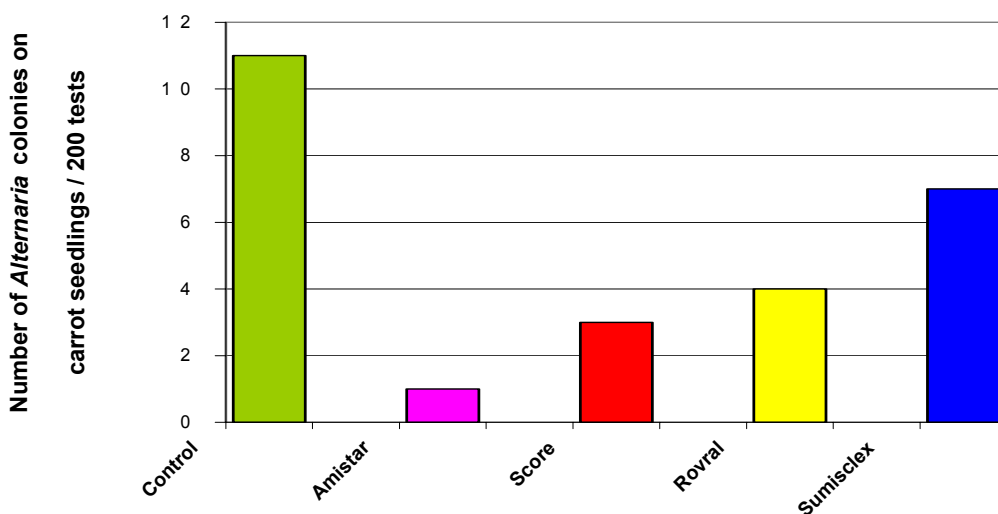


Figure 6. Number of tissue pieces with *Alternaria* on crowns of carrot seedlings treated with various fungicides on 3/5/01 at 8 weeks old. Experiment 2, Virginia 2001

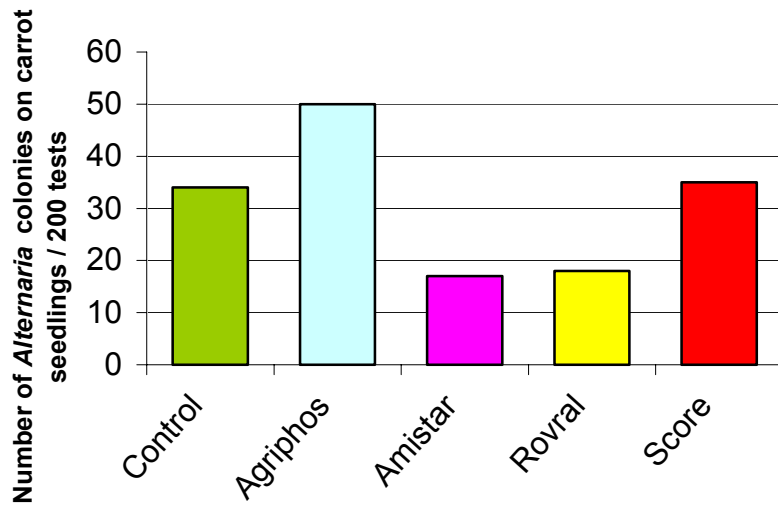


Figure 7. Number of tissue pieces with *Alternaria* on crowns of carrot seedlings treated with various fungicides 8/7/02 at 8 weeks old, Experiment 3, Virginia 2002

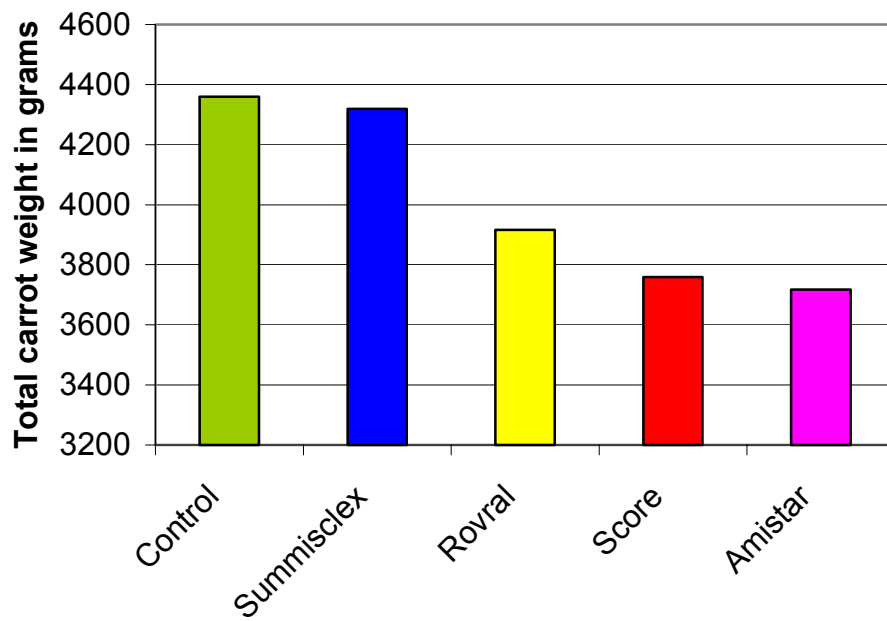


Figure 8. Pre-harvest weights of carrots after fungigation with various fungicides, Experiment 2, Virginia.

FUNGICIDE EVALUATION TO CONTROL FOLIAR INFECTION

To evaluate the control of *A. radicina* leaf infection, the fungicides Amistar, Rovral, Sumisclex and Score were applied to potted eight-week carrot seedlings. The aim was to determine which fungicide had the best curative effect on foliar infection.

Materials and methods

Pots each with 25 six to eight to week seedlings were sprayed with a conidial suspension of *A. radicina* (10^6 conidia/ml). Pots were enclosed in plastic bags for 48 hours to maintain 100% RH and provide conditions conducive to infection. After the bags were removed the fungicides were applied as a foliar spray at the rates shown in Table 4 and repeated 7 days later. Control plants were sprayed with water only. Disease levels were assessed 7 and 30 days after the final fungicide application by counting the numbers of leaves per plant with lesions. Each treatment was replicated five times.

Results and discussion

The percent of leaves per plant with lesions was only 3% and 4% after 7 and 30 days respectively on plants sprayed with water, compared to 68% and 78% on the inoculated plants (Fig 9). All fungicides reduced the level of leaf infection when applied 7 days after artificial infection. In all treatments, infection had increased at 30 days compared to 7 days after inoculation, however in the Rovral and Amistar treatments the levels at 30 days were still significantly lower than the untreated control.

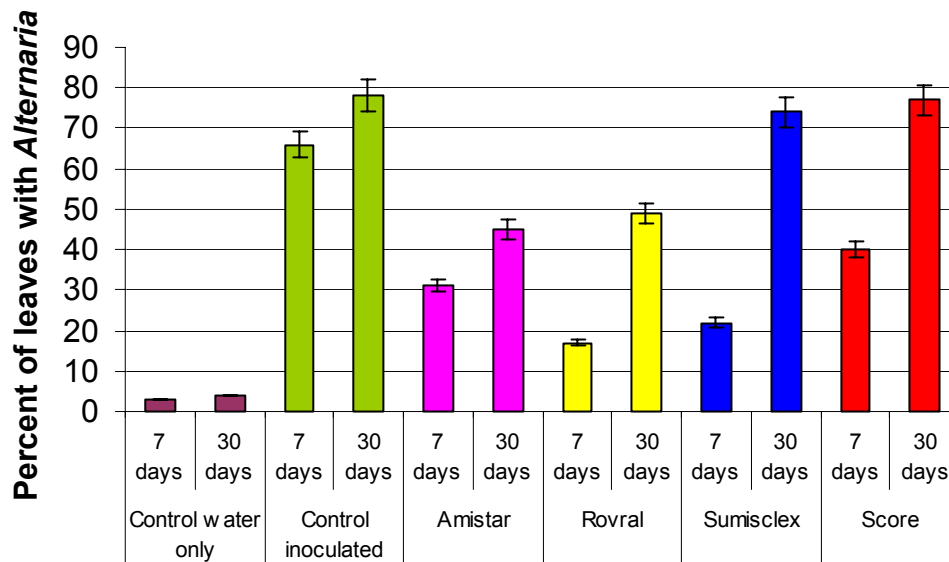


Figure 9. Effect fungicides applied twice after inoculation on leaf infection by *A. radicina*, assessed 7 and 30 days after the second fungicide application

Foliar sprays of Amistar, Rovral and Sumisclex provided low levels of control of artificial foliar infection by *A. radicina*. Similar findings have been reported in Europe and America, as where the leaf canopy becomes dense, effective coverage is difficult (Santos et al., 2000). The efficacy of chemical sprays, resistant cultivars and drip-irrigation systems

in combination provided a more acceptable control of *Alternaria* leaf blight than any one method (Ben-Noon, et al., 2003).

FUNGICIDE TREATMENTS FOR CARROT SEED PRODUCTION

A. radicina is commonly introduced into carrot production areas by using contaminated seed. Nine fungicides were tested to determine if they could be used to reduce the level of *Alternaria* infestation in seed crops. These included: BAS 510 (Filan), BAS 500 (Cabrio), Amistar, Dithane, Score, Rovral, Bravo, Sumisclex and Scala.

Materials and methods

Five secondary umbels per treatment were sprayed with the fungicides at recommended rates shown in Table 4. After 7 days the same umbels were inoculated by spraying with a conidial suspension of *A. radicina* (9×10^4 conidia/ml) and each umbel enclosed in a plastic bag for 48 hours to provide conditions conducive to seed infection. A further five secondary umbels per treatment were sprayed with fungicides at 3 and 7 days after fungal inoculation and bagged for 48 hrs. One month after the final fungicide treatments umbels were harvested, dried, sieved and cleaned. Batches of 250 seeds for each treatment were plated onto ARSA selective media and the percent of seeds with *A. radicina* recorded after 10 days incubation at 30°C.

Results and discussion

Artificial inoculation of umbels resulted in 87% of seed infected with *A. radicina*. All fungicides controlled *Alternaria* when applied before infection. BAS 510 (Filan) provided the best control resulting in levels of 6.5% infection when applied 7 days before infection (Fig 10). When fungicides were applied 3 days after inoculation BAS 500 (Cabrio) and BAS 510 (Filan) provided the most control, but other fungicides were also effective (Fig 11). All fungicides were least effective when applied 7 days after infection (Fig 12).

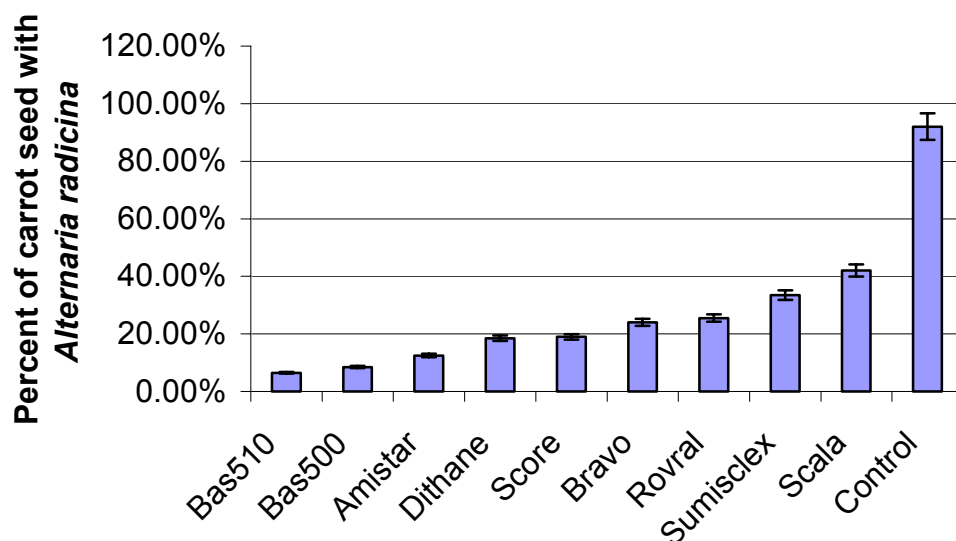


Figure 10. Effect of fungicides applied 7 days before inoculation on the control of *A. radicina* seed infection.

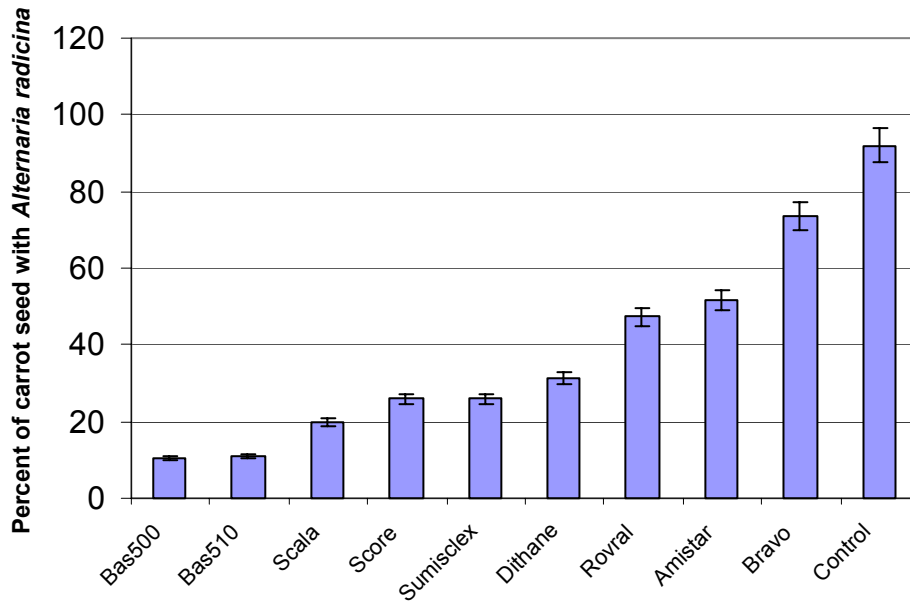


Figure 11. The effect of fungicides applied 3 days after inoculation on the control of *A. radicina* seed infection.

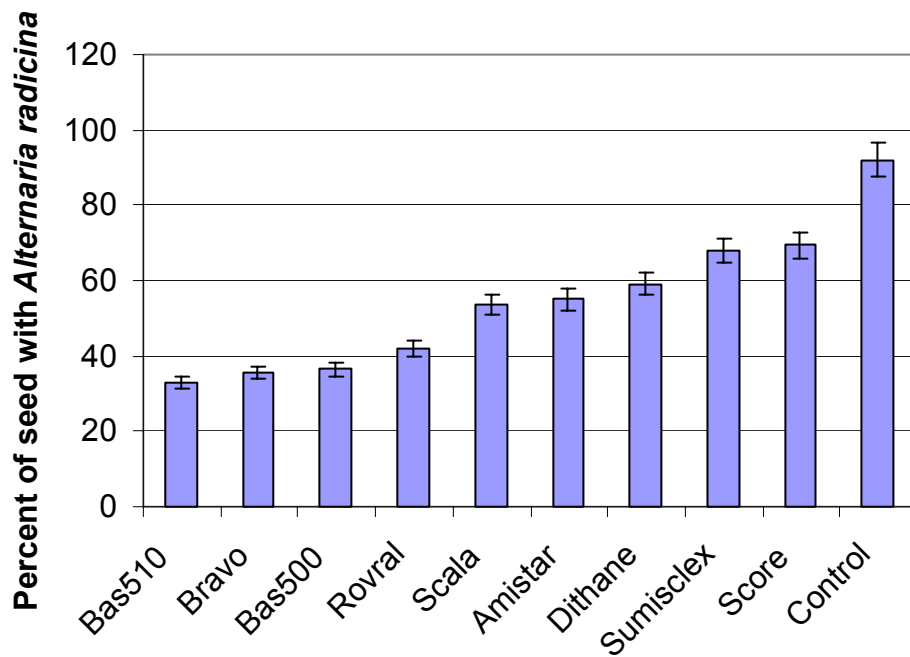


Figure 12. The effect of fungicides applied 7 days after inoculation on the control of *A. radicina* infection.

FUNGICIDE SENSITIVITY TESTS

Isolates of *A. radicina* and *A. carotiincultae* were examined in the laboratory for their sensitivity to commercial fungicides used for seed treatments.

Materials and methods

The *Alternaria* isolates tested were obtained from carrot seedlings, harvest carrots, soil and seed (Table 5). Growth of each isolate was measured on potato dextrose agar amended with 0.05-500mg/L iprodione (Rovral Aquaflo®) and 0.2-2000mg/L thiram, following methods by Biggs (1994).

Fungal discs 6 mm diam. from 14 day-old cultures were placed mycelial surface down onto the amended agar plates using 4 plates per treatment. Two measurements of colony diameters were made at right angles to each other after 7, 10 and 14 days at 25°C. Means were expressed as a percentage of inhibition for each isolate in relation to growth on the unamended media.

Table 5. *Alternaria* spp isolates tested for fungicide sensitivity

Fungal isolate	Origin	Source
<i>A. radicina</i> (Thomson F. Musolino)	Virginia, SA	8 week carrot seedlings
<i>A. radicina</i> (Stephano)	France	Commercial seed with fungicide coat Cultivar: Stephano
<i>A. radicina</i> (India)	India	Commercial seed without fungicide coat
<i>A. radicina</i> (32)	Paringa, SA	Reject post-harvest carrots
<i>A. carotiincultae</i> (98)	Kybybolite, SA	Post-harvest carrots in cold storage
<i>A. carotiincultae</i> (Applebee)	Virginia, SA	Soil from carrot crop

Amistar, which is to be registered for minor use on carrots in SA, was also tested using *Alternaria* conidia to obtain baseline sensitivities. Conidial germinations tests of isolates in Table 5, with the exception of *A. dauci*, were measured using water agar plates amended or not with 0.001-10mg/L Azoxystrobin (Amistar) and salicylhydroxamic acid (SHAM) using methods outlined by Olaya (1998).

Two replicate conidial suspensions of 1×10^4 /ml were added to the surface of either water agar or agar amended with azoxystrobin/SHAM. Conidial suspensions of 50 µl were spread on the agar surface with a glass rod, dried before closing the plate and incubated under continuous light at 20°C for 4 hours. The number of germinated conidia out of a population of 200 was determined after 4 hours, and results expressed in percent inhibition of germination.

Results and discussion

Growth of most *A. radicina* isolates was inhibited by 80% or more at concentrations of 50 to 500mg/L iprodione (Fig 13). The one exception was the isolate from carrot seed which was inhibited by 45% and 64% at 50 and 500mg/L iprodione respectively, indicating resistance. This isolate was from commercial seed from Europe, showing that resistance can be imported with the infected seed and seriously reduce the effectiveness of the fungicides on crops grown from that seed. The crop had most likely been treated with Rovral during the production of the seed, so resistance originated from over exposure to the fungicide either during growth of the crop or on the seed from the seed coat. In the USA, Biggs (1994) has reported similar low levels of inhibition for *A. alternata* isolates from apples that were dipped in iprodione.

Growth of *A. carotiincultae* was inhibited by over 81% at concentrations of 50 to 500 mg/L iprodione (Fig 14), with the soil isolate (Applebee) being slightly less sensitive.

Growth of only one *A. radicina* isolate (Thompson F. Musolino) was inhibited by 100% at a concentration of 200mg/L thiram (Fig 15). All other isolates were inhibited by 69% to 75% at a concentration of 200mg/L thiram and 85% or more at concentration 2000mg/L thiram. Both *A. carotiincultae* isolates were inhibited 90% or more at concentrations of 200 to 2000mg/L thiram (Fig 16).

Conidial germination of most *A. radicina* isolates was inhibited by 97% at a concentration of 10mg/L azoxystrobin (Fig 17). The one exception was the isolate from 8-week carrot seedlings (Thompson F. Musolino), which was inhibited by 77% at 10mg/L. Both *A. carotiincultae* isolates were inhibited by 97% at a concentration of 10mg/L azoxystrobin (Fig 18).

Testing of the sensitivity of isolates of *A. radicina* to azoxystrobin is continuing to obtain good base line data to detect future shifts in sensitivity of the fungus once it is used commercially.

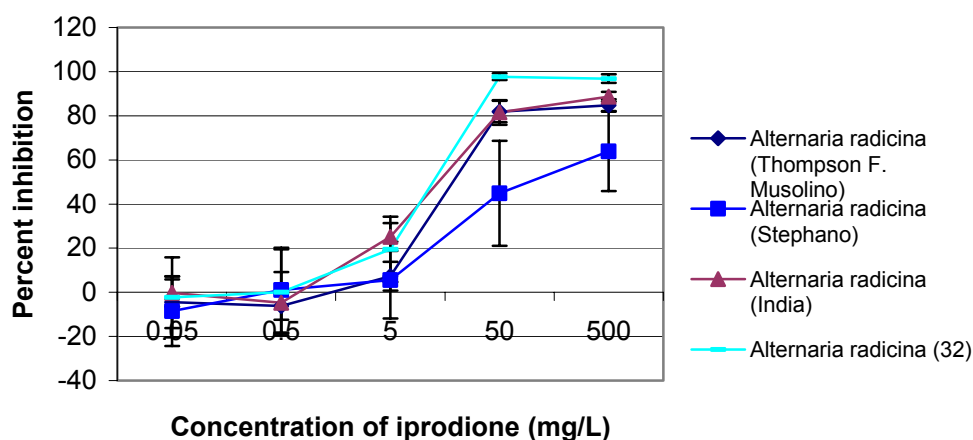


Figure 13. Growth of *A. radicina* isolates in the presence of iprodione

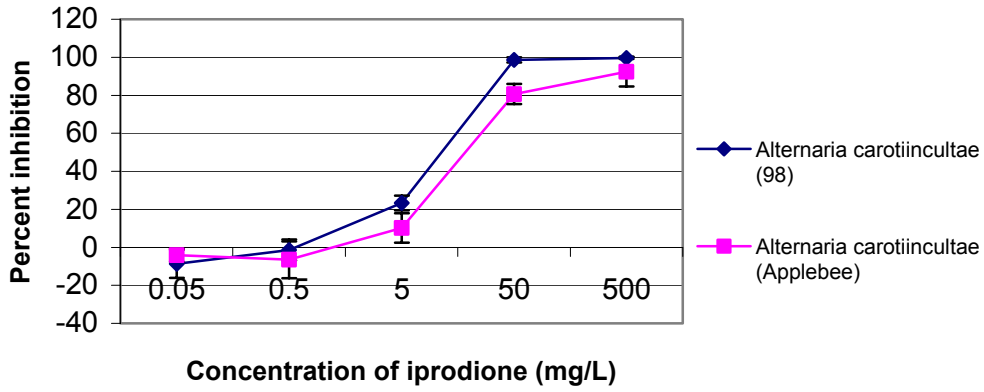


Figure 14. Growth of *A. carotiinculatae* isolates in the presence of iprodione

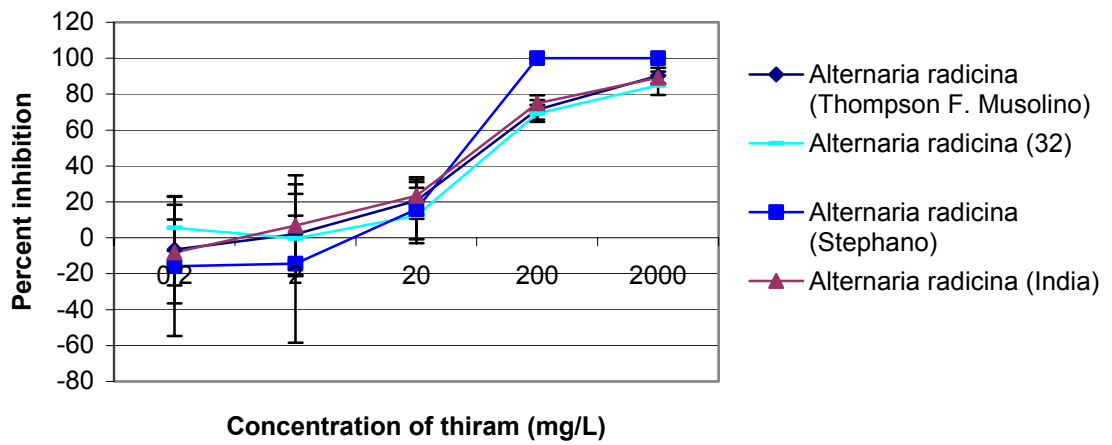


Figure 15. Growth of *A. radicina* isolates in the presence of thiram

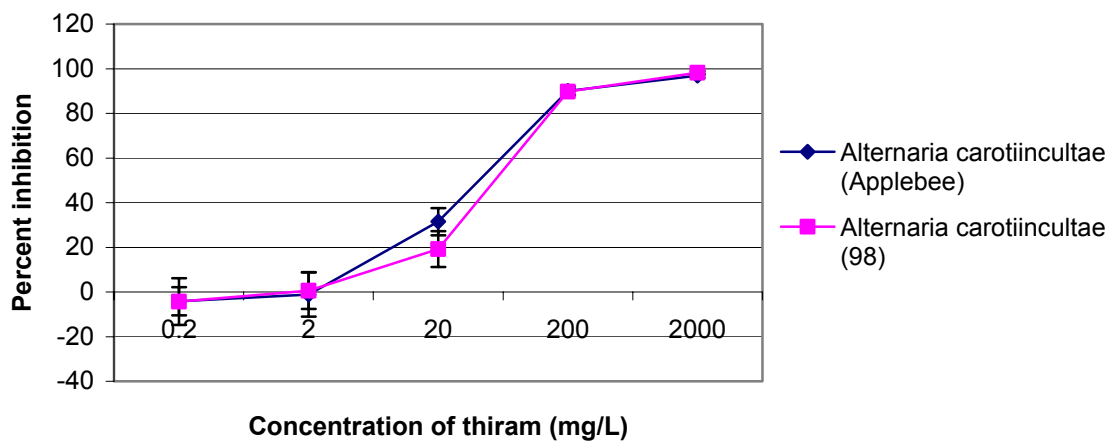


Figure 16. Growth of *A. carotiinculatae* isolates in the presence of thiram

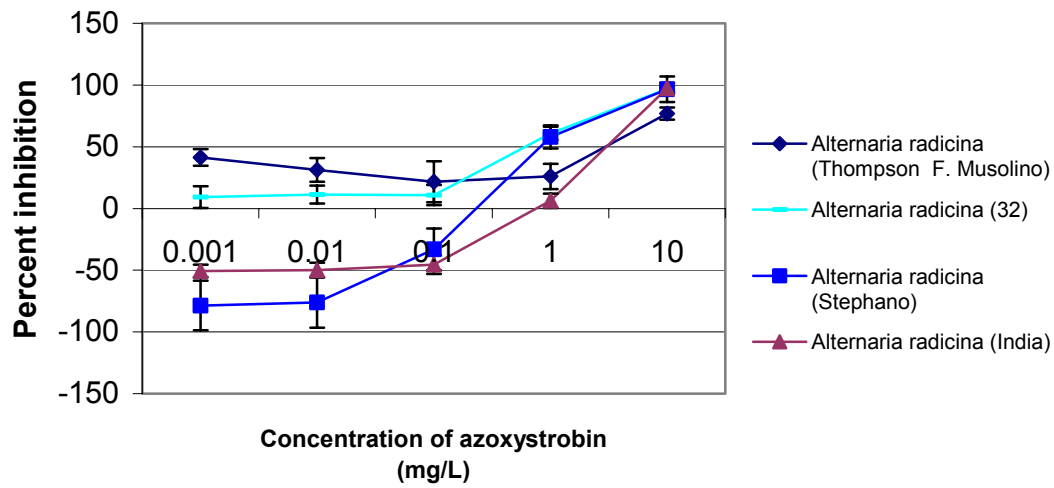


Figure 17. Conidial germination of *A. radicina* isolates in the presence of azoxystrobin

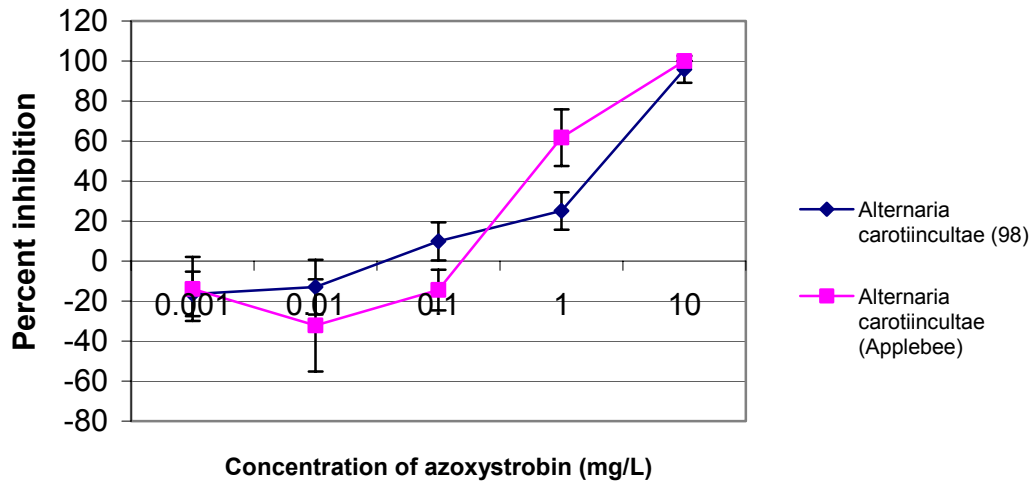


Figure 18. Conidial germination of *A. carotiincultae* isolates in the presence of azoxystrobin

EFFICACY OF SEED TREATMENTS TO CONTROL SEED-BORNE INFESTATIONS OF *A. RADICINA*

Coles and Wicks (2003) showed that many lines of commercial carrot seed were infected with *A. radicina* and *A. dauci*. Of the 19 lines tested, 18 were infected with *A. radicina* with the highest infestation level of 35% occurring on imported seed. *A. radicina* at levels between 0.2 and 14% was also found in 11 of 16 seed batches treated with thiram and or iprodione. Isolates of *A. radicina* from seeds were pathogenic to carrot seedlings and carrot discs. *A. dauci* was also isolated from a number of seed samples, with levels of infection ranging from 0.1-0.3%. A commercial fungicide coated seed lot, Stephano, coated with a mix of iprodione, metalaxyl and thiram, showed infestations of 1% *A. radicina* and 1% *A. dauci*.

It has become evident that fungicide coatings are not always effective in eliminating seed-borne disease. Because of the high levels of seed infestation in some seed lots, various seed treatments were evaluated as a means of controlling seed-borne infections.

Treatment of seed with low levels of *A. radicina* infestation

Materials and methods

To determine the most effective method to control seed borne infestations, treatments were applied to commercial carrot seed where 12% were naturally infected with *A. radicina*. The seed was treated with either a hot water soak at 50°C for 30 mins, potassium permanganate soak (10g/L) at 22°C for 30 mins or steaming at 51°C for 30 mins. The steaming treatment was applied, using a purpose built commercial machine designed to apply steam air mixtures to small batches of seed of ornamental plants. Control batches were not soaked.

All treated seed was dried to the same pre-treatment weight, kept on sterile moist filter paper with 2.4ml of sterile H₂O for 24 hrs then frozen for 16 hrs at -20°C to arrest germination. 50 seeds per plate were evenly spaced over the ARSA agar and incubated at 30°C for 10 days. The plates were examined under 40x magnification for the presence of *Alternaria* on the seeds as previously described. Standard seed germination tests were carried out on 200 seeds per sample at S.A. Seedlab using standard germination tests.

Results and discussion

The results show that a seed soak in hot water at 50°C for 30 mins or treatment with steam completely eliminates *Alternaria* from infested seed (Fig 19). However the steam treatment reduced germination by 11%, whereas the hot water treatment reduced germination by only 4%. Seeds soaked in a 1% solution of potassium permanganate at 22°C reduced seed borne *Alternaria* by 75%, but did not eliminate the infestation completely.

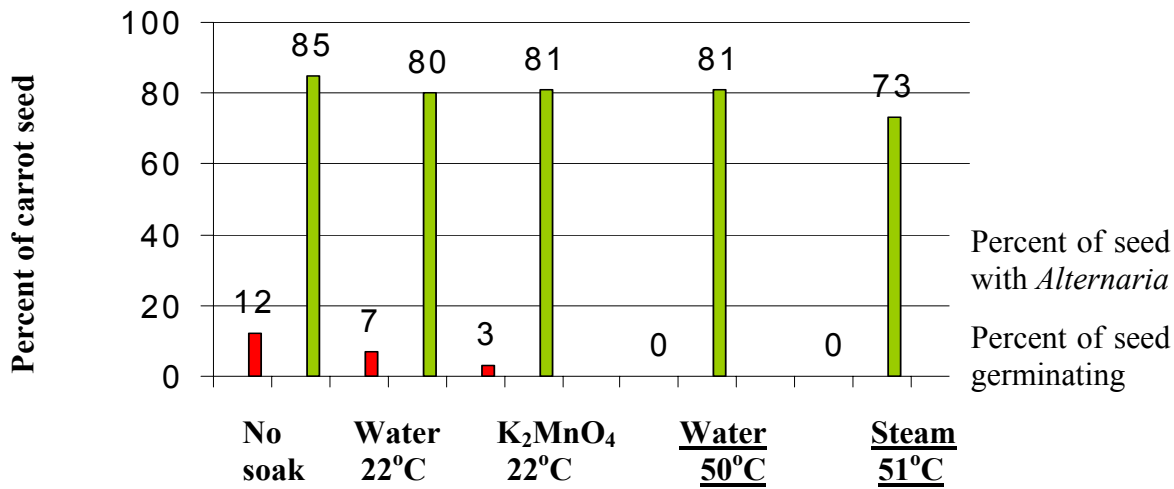


Figure 19. Germination and recovery of *A. radicina* from heating infested carrot seed subject to Potassium permanganate, hot water soaks or steam treatments.

Treatment of seed with high levels of *A. radicina* infestation

Further experiments were carried out with carrot seed artificially infected with *A. radicina* that resulted in an 82% level of seed infestation. In this experiment various treatment with 6% hydrogen peroxide were compared with a hot water (50°C) treatment for 10 mins.

Materials and methods

Seed batches of at least 1.5g/ treatment were either soaked in water for 10 minutes at room temperature (RT) 22°C, or 50°C, treated with 6% hydrogen peroxide, for 10 min at RT, or 10 min at 50°C and 24 hrs at RT.

The levels of *A. radicina* infestation on the treated seeds were measured as previously described, and percent germination calculated on 200 seeds/treatment.

Results and discussion

Infestations of *A. radicina* were reduced from 82% for dry seed to 34.3% in seed soaked in water for 10 min at RT (Fig. 20). Further reduction was achieved with water soaks of 50°C, which reduced infestation to 9.8%. The most effective control of *A. radicina* infestation was achieved with hydrogen peroxide at RT after 24 hrs, but even this did not completely eradicate the infection.

The high level of *A. radicina* infestation reduced germination of untreated seed to 6% while all other water and hydrogen peroxide soak treatments had germination levels around 14% (data not shown).

Reduction in seed-borne *Alternaria* disease levels by heat treatments has been widely promoted since Shimmer (1953) recommended 50°C soaking for 18 mins. However the practice has not been taken up because of seed drying and planting difficulties associated with moist bulk materials. The reduction of *Alternaria* infestation in infested seed after 10 mins soak in water at RT is probably related to multiplication of naturally occurring antagonists in the infested seed. The low level of seed germination in the highly infected seed was a result of high percentage infestation by the artificially inoculated pathogen *A. radicina*.

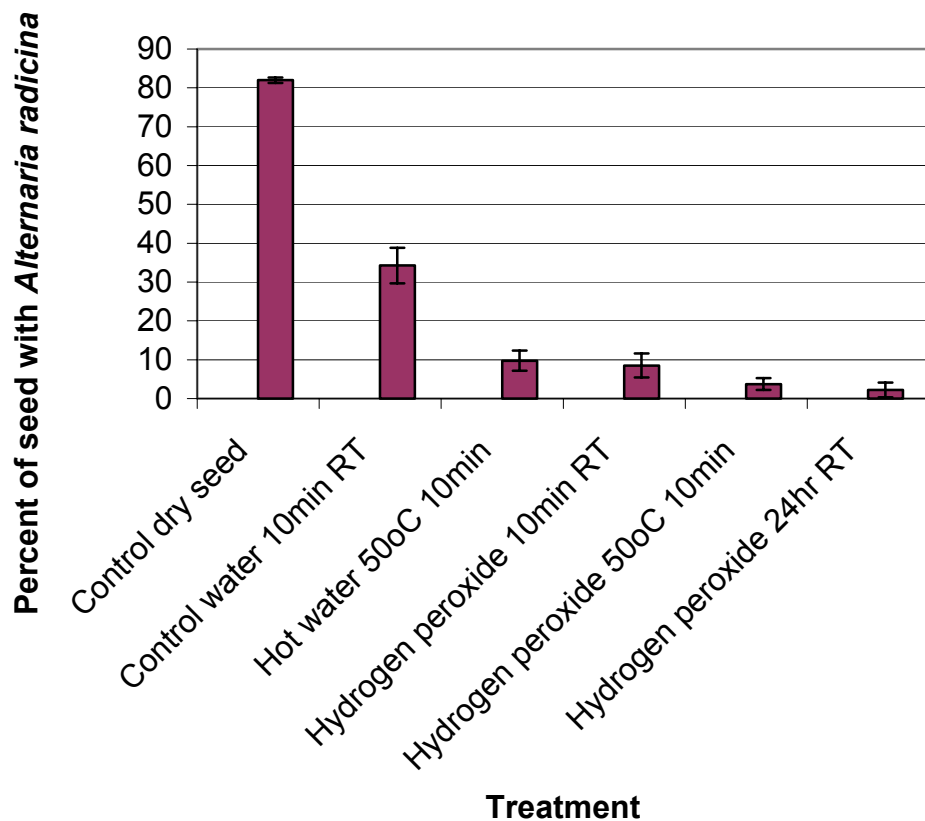


Figure 20. Recovery of *A. radicina* from artificially infected seed treated with hot water and hydrogen peroxide.

Bio-priming carrot seed with *A. radicina* antagonists

An attempt was made to bio-prime artificially infected seed with potential bio-control agents that were isolated from disease free seed.

Materials and methods

Commercial batches of seed free of fungicides were incubated in a standard freezer-blotter method (Pryor et al, 1994). Fungi growing on the seed surface were isolated by picking off mycelium and spores using sterile needles and plating them onto PDA with 200ppm chlortetracycline, and incubating in 12 hrs light and dark for 10 days at RT. Fungi were identified to genus from spores using a standard mycological key (Barnett, 1965).

Antagonistic fungi were assessed against *A. radicina* following methods of Knudsen (2003). Three isolates were obtained from a seed batch provided by South Pacific Seeds which had a level of 0.4% *Gliocladium* (A, B) and *Penicillium* spp (C). Spore suspensions were harvested from the cultures of these fungi using a sterile spatula and sterile water. Suspensions were diluted to 10^7 conidia/ml and seed that had been artificially pre-infected with *A. radicina* was soaked separately for 10 mins in antagonists A,B and C and a mixture of A,B,C. Controls were soaked in sterile water. Three hundred seeds per treatment were dried at 30°C for 24 hours and placed in petri dishes with moist filter paper, incubated at 24°C for 10 days under 12 hours light and dark. Individual seeds were examined for *A. radicina* conidial formation using a binocular microscope at 40X magnification. Seed germination for each treatment was assessed after 21 days.

Results and discussion

Dry seed had a higher level of *A. radicina* infestation (82%) compared to seed soaked in water for 10 min at RT (17%), Fig 20. All the seed soaks with *Gliocladium* and *Penicillium* spp. reduced levels of *A. radicina* infestation to 2% - 3%. Seed germination improved from 6% for dry seed to 14% for the water soaks and 21% to 24% for the antagonist soaks (Fig 21).

In untreated carrot seed naturally occurring antagonists of *A. radicina* occur within the seed coat. Some suppress pathogen development. Knudsen (2003) discussed the effect of bio-priming carrots seed with antagonists such as *Clonostachys/Gliocladium* spp., which multiply on the seed coat in the presence of moisture. Application of the bio-priming procedure was achieved by coating carrot seed with a peat-bran formulation of four selected antagonists. This improved plant germination in a bioassay in which the results of seedling emergence were at the same level as an iprodione treatment (Knudson, 2003)

Storage and application of antagonists in the field requires a stable substrate that protects against drying and UV light. In vitro tests showed high-silica "Maidenwell Diatomite" was an ideal inert growing medium. It had a 150% weight water absorbency capacity and was found to be an ideal carrier. It could be easily inoculated, dried and applied in the field with a carrot seeder.

In vitro tests showed *A. radicina* isolates inoculated onto PDA side by side with the antagonists did not grow after 12hrs light dark incubation for 10 days, either at RT or 30°C.

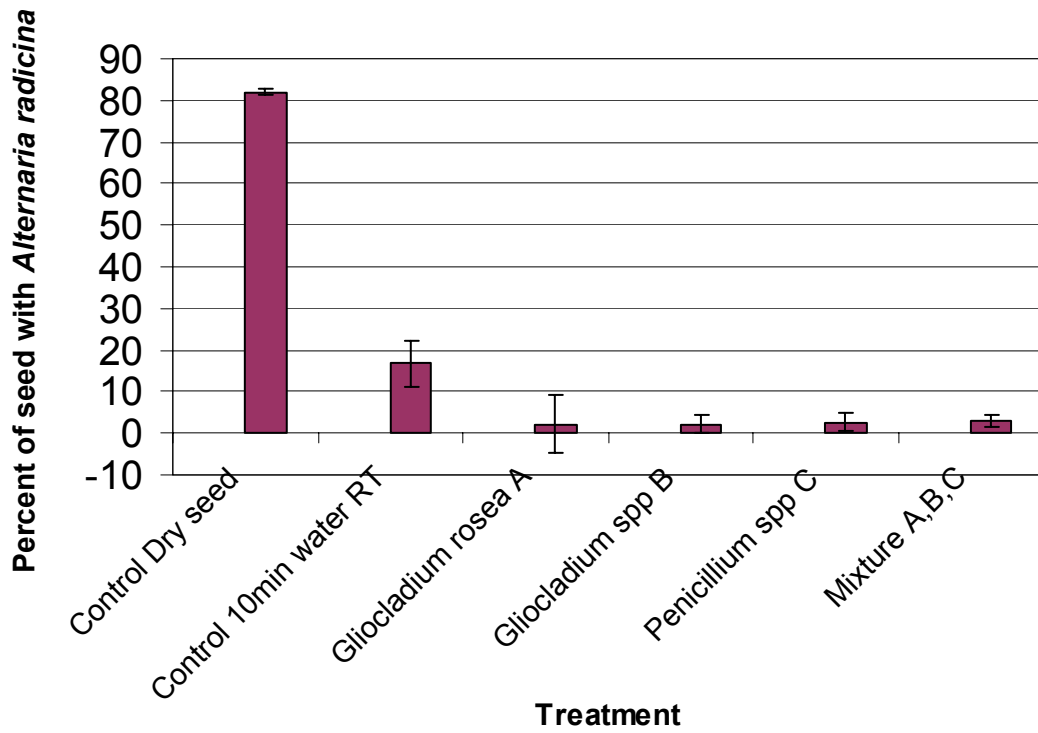


Figure 20. Percent of seed with *Alternaria radicina* after bioprimering with antagonists.

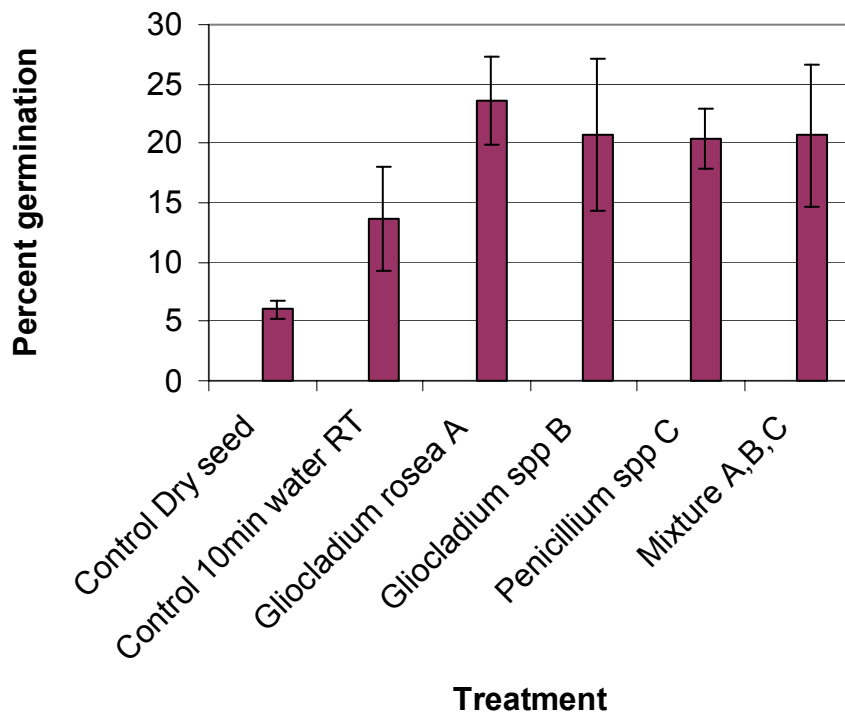


Figure 21. Percent germination of artificially infested seed after bio-priming

COMPARATIVE RESISTANCE OF CARROT CULTIVARS TO INFECTION BY *A. RADICINA*

Cultivars resistant to *A. radicina* infection can provide improved control for the management of *Alternaria*. Studies in Europe, Asia and America have identified resistance in Amarak, Berlikum, Emperor, Nantes and Chantenay-type carrots (Pryor et al., 2000). However, previous Russian research had shown that no carrot cultivars were immune to infection by *Alternaria* spp., (Vlasova et al., 1988).

In America commercial hybrid cultivars showing the highest resistance to artificial shoulder infection were the Emperor Nantes types: Caro-pack, Gladiator, Panther, Caro-spike, Six-pack and Jaguar. Those with the lowest resistance were also commercial varieties in the Nantes, Emperor and Chantenay types, which included: Orlando Gold, Scarlet Nantes, Nogales, Royal Chantenay and 2003. Cold storage tests also showed the cultivars Caro-pak and Pather had the highest resistance while Nogales and Royal Chantenay had the lowest resistance to infection by *A. radicina* (Pryor et al., 2000). In this project glasshouse studies were undertaken to evaluate the susceptibility of a number of carrot cultivars to infection by *A. radicina*.

Materials and methods

Twenty-eight cultivars were obtained from commercial sources, growers and seed companies. Most were planted commercially in SA and Vic or imported from overseas for commercial use. Others such as the All-seasons, Amsterdam and Golfball were used only in specialty bunch markets overseas. The various cultivars included most of the carrot types and were tested in a green house for their resistance to foliar infection by *A. radicina*. Conidial suspensions of 10^6 /ml were sprayed onto the foliage of 6-8 week carrot seedlings with five plants per pot, and four replicates for each cultivar, as described in the general methods for artificial infection of seedlings and fungicide evaluations.

Levels of disease on the seedlings were assessed 30 days after infection using an average disease rating of the area of leaf surface with *A. radicina* lesions for the total number of carrot plants/cultivar, Table 6.

Results and discussion

Although no carrot cultivars are resistant to *Alternaria* infection some of the more resistant varieties are smaller types: All seasons, Amsterdam and Golfball types which fit the Paris Market and Amsterdam Forcing cultivars (Table 6). The more susceptible varieties, often grown commercially in Australia are Nantes, A91, Danvers A8 and Carrisma C. The implication of these findings is that many of the cultivars grown in the temperate region of Australia are moderate to highly susceptible to *A. radicina* infestation.

Hybrid Nantes types most commonly grown in Australia for the fresh market, juicing and export also have low resistance to *A. radicina*. Further research is required on the selection of cultivars that show higher levels of disease resistance to *Alternaria*. Overseas work has shown that an integrated disease management program for foliar infection by *Alternaria* was greatly enhanced by using resistant varieties in conjunction with modified drip

irrigation. This practice also reduced the frequency of fungicide foliar applications (Ben-Noon et al., 2003).

Table 6. Commercial carrot cultivars and resistance to foliar infection by *A. radicina*

Cultivar	Mean disease level*	Number of plants	Standard deviation
All season	1.20	25	0.58
Amsterdam	1.40	25	0.96
Golfball,	1.45	33	0.67
Imperator,	1.55	38	1.03
Kuroda, US	1.56	25	0.82
Kuroda,A82	1.63	32	0.66
Manchester	1.77	26	0.95
Kuroda,A80	1.92	36	1.18
Space save	1.93	14	0.62
Flame CR28	1.94	34	1.15
Yates, Top	1.96	26	0.66
Chantenay	2.14	36	1.05
Purnong, Y	2.33	27	0.78
Flakee,A83	2.50	20	0.89
Corona CR	2.55	22	0.67
Fairbanks	2.57	30	0.63
Flakee, A8	2.60	43	0.69
French Hav	2.77	30	0.77
Maroon Ind	2.86	14	1.10
Havana, Di	2.96	23	0.47
Havana	2.96	25	0.89
Nantes, A8	3.04	24	0.86
Stephano	3.08	26	0.80
Japan,Mojo	3.10	10	0.32
Japan,Yoda	3.13	24	0.85
Nantes,A91	3.48	31	0.81
Danvers,A8	3.82	17	0.73
Carrisma,C	3.88	16	0.72

*Mean disease level ratings: 0 = no lesions, 1 = 5% area of leaves with lesions, 2 = 25% area of leaves with lesions, 3 = 50% area of leaves with lesions 4 = 90% area of leaves with lesions level of disease.

SUMMARY:

- This project has shown that *Alternaria* and other fungal pathogens are being introduced from overseas in infested seed.
- *Alternaria* has become established in most carrot growing areas of SA by the planting of infested seed.
- Soil fumigation with Metham and Telone reduced levels of *Alternaria* and beneficial *Streptomyces* spp in infested soils, however *Alternaria* levels increase near harvest and carry over into the next crop.
- Fungicide drenching is an effective method of reducing the levels of crown and petiole infections on seedlings. Drenching also reduces levels of soil borne *Alternaria*.
- Fungicide drenches of seedlings has shown that Amistar and Rovral Aquaflo® provided early control of *Alternaria* infections.
- *Alternaria* infections of carrot flower umbels were controlled by BAS 510 (Filan®) and BAS 500 (Cabrio®).
- Populations of *A. radicina* with reduced sensitivity to iprodione (Rovral) were detected in seed coated with the fungicide.
- Fungicide seed coatings do not always successfully control *Alternaria*.
- *Alternaria* infestations of seed can be successfully eliminated by hot water soaks, at 50°C for 30 min or steaming at 51°C for 30 min.
- Potassium permanganate (1%) and hydrogen peroxide (3-6%) soaks at RT provided good control of *Alternaria* seed infestations.
- Fungal antagonists of *Alternaria* isolated from carrot seed provided high levels of control of infested seed after 10 min soaks at RT.
- Carrot cultivars showing some level of resistance to *Alternaria* infection were Amsterdam, Paris Market and various Imperator, Kurodo types.
- Susceptible cultivars were those often used commercially in WA and SA such as Stephano, Carrisma, Havana and specific Nantes and Danvers types.

TECHNOLOGY TRANSFER

Direct contact with growers, and via discussion with seed companies and other researchers have released the research findings of this project. Findings have also been sent to Vegetable Industry Development Officers within Australia. In addition four newsletters outlining the extent and cause of the problem were issued in February 2000, July 2001 and September 2002 (Appendix 2). These newsletters were distributed to carrot growers and included on the SARDI web site.

Articles and the Management of *Alternaria* Blight in Carrots have been included in The Grower Magazine, PIRSA: Farm Gate, and the Vegetable Platter (Appendix 2)

Papers, posters and conferences:

(1) Scientific Refereed Publications

Coles RB and Wicks TJ 2003 The incidence of *Alternaria radicina* on carrot seeds, seedlings and roots in South Australia. *Australasian Plant Pathology*. **32**, 99-104.

(ii) Conference Abstracts

Coles RB, Walker GE and Wicks TJ (2003) The effects of Metham and Telone on soil levels of *Alternaria radicina* and *Streptomyces* spp in a carrot crop. In *8th International Congress of Plant Pathology* Christchurch New Zealand 2-7 February 2003 p71.

Coles RB and Wicks TJ (2003) The occurrence of the carrot pathogen *Alternaria radicina* on common fumitory weeds in South Australia. In *8th International Congress of Plant Pathology* Christchurch New Zealand 2-7 February 2003 p53.

Coles RB and Wicks TG (2001) New Fungal Pathogens of Carrots in South Australia. In *13th Biennial Conference Cairns*, 24-27 September p103.

(iii) Newsletters/Magazine articles

Carrot News, Issue 1. February 2001. Causes of carrot seedling losses in SA

Carrot News, Issue 2. July 2001. Causes of carrot seedling losses in SA

Carrot News, Issue 3. February 2002. Managing *Alternaria* Blight in Carrots

Carrot News, Issue 4. August 2003. Carrot Virus Y update in South Australia

Good fruit and Vegetable, September 2003. Carrot seed research update

Vegetable platter, September 2003. Carrot seed research update

PIRSA: OPENGATE December 2002 Carrot fungus in hot water p11

RECOMMENDATIONS-SCIENTIFIC AND INDUSTRY

MANAGEMENT STRATEGIES:

Control of Alternaria blight in carrot seed

- Seed monitoring for disease levels of *Alternaria* are recommended for all carrot seed lines produced locally and imported from overseas.
- Seed lines with high levels of *Alternaria* infestation should be treated with hot water soaks, at 50°C for 30 mins or steaming at 51°C for 30 mins.
- Seed lines with low levels of *Alternaria* infestation can be treated with either Potassium permanganate soaks (10g/L) at 22°C for 30 mins or 6% Hydrogen peroxide soaks at 50°C for 10 mins or alternatively 22°C for 24 hrs.

These procedures would eliminate the need for fungicide seed coats and some would enable commercial seed to be certified for organic grower use.

Fungicide drenches to control Alternaria blight in 6 to 8 week seedlings

- Drenches with Amistar 450g/Ha and Rovral Aquaflo (1L/Ha) are recommended for the control of crown and petiole infections on 6 to 8 week old seedlings.
- Rovral drenches also reduce *A. radicina* levels in the soil

Fungicides treatments for carrot seed production

- *Alternaria* infection of umbels were controlled by BAS 510 (Filan) 450g/Ha, BAS 500 (Cabrio) 250 ml/Ha applied either before or after infection. Amistar was effective if applied before infection.

Fungicide treatments to control foliar infection

- Foliar application of Amistar (450g/Ha) and Rovral (1L/Ha) control of *A. radicina* in shade house pot trials.

Management practices to control Alternaria outbreaks in subsequent carrots crops

- Planting rotation crops that are non-hosts for *Alternaria* such as barley, broccoli or other brassicas.
- Green mulching of brassica crops when 15% flower set has occurred, followed by irrigation greatly increases the penetration of the bio-fumigants (isothiocyanates) into the soil.
- Avoid the regular use of soil fumigants, Telone or Metham. These fumigants may reduce soil levels of micro-organisms that are antagonistic to *Alternaria* spp.

- Overseas work has shown that drip irrigation instead of overhead sprinklers controls water splash dispersal of *Alternaria* spores.

FUTURE WORK

- Trial carrot cultivars found to be partially resistant to *Alternaria* in commercial crops and compare them to non-resistant cultivars.
- Establish a series of Brassica rotation trials to reduce soil borne *Alternaria* levels in field sites where *Alternaria* levels are high
- Involve commercial seed producers to evaluate fungicides such as azoxystrobin, pyraclostrobin and boscalid fungicides to control seed-borne *Alternaria* and *Cercospora carotae* infestations.
- Trial bio-priming of commercial carrot seed with *Alternaria* antagonistics to control seed-borne pathogens
- Trial a production and application of *Alternaria* spp antagonists for commercial seed lots
- Develop a commercial soil inoculum of antagonists for control of soil-borne *Alternaria* spp.
- Undertake fungicide evaluation trials in relation to timing and frequency of applications in field and seed crops

Additional Commercialisation

Residue trials were undertaken in two carrot-growing regions in SA in conjunction with a CPA (Study Plan No: azoxystrobin AVG876). This is part of a national study for registration of Amistar use in carrot disease control.

The CPA is currently establishing Rovral for minor use on carrot seedlings to be used in resistance management of *Alternaria* based on information obtained in Virginia fungicide trials in 1999 to 2001.

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APPENDIX 1.

Recipe for *Alternaria radicina* selective agar (ARSA) (Pryor et al., 1994)

ARSA is an extremely useful media to isolate *Alternaria* spp., *Ulocladium* spp and *Stemphylium* spp. from soil or seed (. The media inhibits the growth of many saprophytic fungi and allows easy identification of *A. radicina*. It also allows the growth of *Streptomyces* spp, which can be measured in the soil after fumigation and fungigation treatments. Growth of *A. radicina* on ARSA is distinctive and best viewed by examining the under surfaces of the plates. Both prolific and sparse hyphal growth from inoculum points is dense, thick black to olive-black and extend downwards into the media. Sparse growth of *A. radicina* on ARSA often appears as one to several irregularly branched hyphae. The saprophytic fungi that do grow on ARSA often appear hyaline to olive-brown their hyphae are much thinner than those of *A. radicina*. They did not always deeply penetrate the agar medium.

This recipe has been modified by excluding the herbicide 2,4-D and substituting the fungicides: 100mg a.i. triadimefon (200mg of Bayleton 50WP/L) and 106mg a.i. thiabendazole (116.6mg of Tecto 90WP/ L).

The media is made up in three separate parts:

A.

16g of Bacto agar
1.0g of KH₂PO₄
1.0g of KNO₃
0.5g of KCL
0.5g of MgSO₄
500ml H₂O

B.

5.0g of Sodium polypectate=polygalacturonic acid Sodium salt (Sigma P3850)
500ml H₂O

C. (Not autoclaved)

The following antibiotic and fungicide mix can be added to 10ml of sterile water with some glass beads and shaken to break up the small lumps of Bayleton and Tecto

50mg chlortetracycline HCl
50mg streptomycin sulphate
4mg a.i. of dicloran (5mg of Botran 75WP) **NB this must be dissolved in 5ml hot 100% ethanol**
100mg triadimefon (=200mg of Bayleton 50WP)
106mg of thiabendazole (=116.6mg of Tecto 90WP)

PARTS A AND B ARE AUTOCLAVED SEPARATELY, COOLED TO 50°C THEN COMBINED AND C ADDED.

APPENDIX 2.

Paper in Australasian Plant Pathology (2003) 32: 99-104.

The incidence of *Alternaria radicina* on carrot seeds, seedlings and roots in South Australia

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^A Corresponding author *Key words:* *Alternaria radicina*, carrot seedlings, disease distribution, *Daucus carota*

Abstract

Surveys of eight carrot growing regions in South Australia conducted from December 1998 to May 1999, showed that poor seedling establishment was associated with high levels of infection by *Alternaria radicina*. Seedling losses were widespread throughout the state and were most frequent between February and April 1999. Up to 47% of seedlings and 88% of mature plants on some properties were infected by *A. radicina*. Isolations from diseased plants showed that *A. radicina* attacked carrots at all stages, causing damping-off, rotting of roots crowns, seedling petioles leaves and crowns of maturing carrots. Up to 70% of mature carrots with crown and shoulder infections were unmarketable. Of the 19 lines of commercial seed tested, 18 were infected with *A. radicina* with the highest infection level of 35%, occurring on imported seed. *A. radicina* at levels between 0.2-14%, was also found in 11 of 16 seed batches treated with thiram and/or iprodione. Isolates of *A. radicina* from seeds or infected seedlings were pathogenic to carrot seedlings and carrot discs. *Alternaria dauci* was also isolated from four seed samples, with levels of infection ranging from 0.1-0.4% and on foliage in three of the eight carrot growing regions.

Introduction

Losses due to poor seedling establishment and damping-off have been reported by Carrot growers in South Australia since the 1970s. This disorder occurs unpredictably, usually during periods of warm humid weather, and has not been successfully controlled by fungicide such as thiram and iprodione applied as a seed coating (Soteros, 1979). Little work has been conducted on the cause of damping-off of carrot seedlings in Australia except in Queensland where *Rhizoctonia* and *Pythium* were implicated (R. G. Obrien Unpublished data). In Western Australia, *Pythium* spp. caused damping-off of carrot seedlings and cavity spot in mature carrots (Davison et al., 1998). A limited survey in the Virginia area of South Australia in 1994 implicated *Alternaria* and *Fusarium* as possible causes of carrot seedling damping-off (T. Wicks, unpublished data), but no pathogenicity tests were carried out to confirm this.

This paper reports on more extensive surveys undertaken to determine the extent of the problem in SA. Studies were also undertaken to determine the cause of seedling losses and to ascertain if infected carrot seed was associated with the problem.

Materials and methods

Survey of carrot plantings

The regions surveyed for carrot seedling disorders are shown in Fig. 1. These include: (1) Virginia, 12 km N of Adelaide, where vegetables have been produced for more than 50 years; (2) the Riverland near Paringa, 220km ENE of Adelaide; (3) the Mallee region near Parilla, 192 km ESE of Adelaide; (4) the mid south east near Kybybolite, 309km SE of Adelaide with an expanding vegetable industry; and (5) the lower South East (SE) near Mount Gambier, 387 km SE of Adelaide where commercial carrot seed is produced. Smaller and more recently established growing regions surveyed were: (6) Ashbourne, in the Adelaide Hills 45 km S of Adelaide; (7) Nuriootpa, in the Barossa Valley 60 km NE of Adelaide; and (8) Blanchetown, near the River Murray, 115 km NE of Adelaide. Soil types in these areas were mainly sandy loams and silts, except for those near Mount Gambier which were volcanic silts and clays.

Carrot plantings from three fields in each of the eight growing regions in SA were surveyed 1998 to 1999 at one to two monthly intervals, depending on the availability of sites during the year. Each area with six to eight week old plantings was sampled six to twelve times. At each sampling time 50 to 100 seedlings with obvious damping-off symptoms, stunting, stem rot, wilting or leaf discolouration were collected. A similar number of healthy plants were collected from adjacent plantings. Isolations were made from at least six of the seedlings taken from one sample that showed obvious disease symptoms on the root hairs, root tip, mid root, hypocotyl region, stem and petiole. Similar isolations were made from six healthy seedlings. Sections of plant material, 1mm thick, were surface sterilised for 10 sec. in 70% ethanol, rinsed in sterile water and surface dried on sterile filter papers. Three replicate sections of each plant were then plated onto (i) *Pythium* selective Corn Meal Agar (CMA), (Davison and McKay, 1998); (ii) Potato Dextrose Agar (PDA with 200 ppm chlortetracycline); (iii) V8 juice agar (200ml juice/L with 2g calcium carbonate and 250 ppm streptomycin) and (iv) water agar (WA, difco bacto-agar with 200 ppm chlortetracycline). Where isolates of *Alternaria* did not sporulate, carrot leaf agar (Strandberg, 1987) was used to promote sporulation (CLA Bacto agar with carrot leaf extract and 250 ppm streptomycin). Plates were incubated at 22°C in the dark for 5 days, followed by 12 hrs light and 12hrs dark and examined at 5, 10 and 21 days. Foliage pieces showing lesions and marginal necrosis were surface sterilised as above and incubated in moist chambers at room temperature. Pieces were examined after two days and fungi that developed were identified from spore dimensions using standard mycological keys (Barnett, 1965; Ellis, 1971).

Distribution of poor establishment

One property with high disease levels in the Barossa Valley was surveyed in detail to determine the distribution of damping-off and stunted seedlings in a carrot planting. Nine paired rows of cultivar Thor and ten paired rows of cultivar Havana seedlings aged 4 to 6 weeks were assessed in an area of 100 m x 44 m. Seedlings were assessed every 2 metre along 100 m of row length. Plant density was rated as high (41-50 plants/m), medium (11-

40 plants/m) or low (0-10 plants/m). In the high densities no obvious damping-off symptoms were present, where as in low and medium densities damping-off and plant stunting was obvious. Isolations were made from healthy and diseased seedlings as described previously. The percentage of disease levels in both cultivars was determined by comparing the density of healthy and diseased plants within the area sampled.

Infection of mature carrot crops

During the survey it was observed that maturing carrots were often damaged around shoulder and crown regions. Preliminary investigations showed that this was probably the result of *Alternaria radicina* Meier, Drechsler & Eddy infection as the fungus was isolated from damaged periderm and phloem tissue. Confirmation that *A. radicina* was the cause of mature crop infection was obtained from dissected diseased tissue. Fragments of carrot from the shoulder and crown areas that contained containing hyphal material produced sporulating colonies of *A. radicina* on PDA. Cross sections of this area showed brown-black hyphae, typical of *Alternaria* spp. ramifying through this tissue. To determine the incidence and severity of this disease in mature crops, five separate properties in three regions were surveyed. Carrots were hand harvested in April and May 1999 by randomly taking 20 samples each of five or more carrots in a zig-zag pattern through the first 200 m of the field. The carrots were washed and rated for damage on the shoulder or crown using a 0 to 4 scale where: 0 = healthy, 1 = some blackening at top of the tap root and one constriction ring, 2 = slight blackening and two or three constriction rings, 3 = moderate blackening and three to four rings, 4 = severe blackening or dark purple colouration and four or more rings (Fig 3). Carrots with a rating 2 or more were considered unmarketable.

Isolation of fungi from commercial carrot seed

Seed samples donated by seed companies and carrot growers or purchased from retailers were tested for fungal infection. The origin of the seed was overseas and Australian. Ten replicate samples each of 50 seeds were selected at random for each batch. A semi selective media, *Alternaria radicina* selective agar (ARSA) and described by Pryor et al.(1994) was adapted to assess seed quality. After 10 days each seed was examined and fungal species were identified using standard mycological keys (Barnett, 1965; Ellis, 1971).

A separate batch of 19 commercial seeds, all previously treated by coating or soaking with fungicides thiram and/or iprodione , were also assessed for *Alternaria* spp. using the method described above.

Pathogenicity tests

The pathogenicity of fungi isolated from diseased plants was determined using carrot discs test modified from (Pryor et al., 1994) and live seedlings.

Carrot disc pathogenicity tests

Mature "Carissima" carrots were assessed for pathogenicity of *Alternaria* spp using a test adapted from Pryor et al., 1994. Eleven isolates of *A. radicina*, two of *A. dauci* and one of *A. alternata* were assessed for their pathogenicity on carrot disc using a modified 0 to 4

scale. The scale was similar to that used in mature carrot disease ratings, where: 0 = no discolouration of the disc, 1 = slight discolouration, 2 slight discolouration with mycelial growth, 3 = grey to black necrosis with some production of conidia and 4 = grey to black necrosis with abundant production of conidia.

Each test was replicated four times. The data were subjected to ANOVA, and the means were separated by the least significant difference (LSD) test.

6-8 week old seedling test

The second method used fresh 6 and 8-week old carrot seedlings of the commercial cultivar "Carrisma" which were dug from the field and washed thoroughly in tap and deionised water to remove soil. Seedlings were placed on surface sterilised aluminium foil sheets in pre-washed plastic trays with pre-moistened absorbent paper. Six seedlings per treatment were inoculated by taking 1.0 x 0.5cm water agar pieces from mature colonies of *A. radicina* and placing the mycelial surface down on to the hypocotyl region near the crown of each seedling. Controls were treated similarly using similar sized pieces of water agar. The trays were enclosed in a clear plastic bag and incubated on the laboratory bench at room temperature for 10 days. The level of disease was assessed by measuring the extent of necrosis from the point of inoculum. Seedlings were also examined with a dissecting microscope to rate infection on and around the point of contact with the infection source. Symptoms were rated on a similar scale to the carrot disc pathogenicity test. Fungi causing blackening, or soft decomposition of the hypocotyl region, or death of the upper stem and petioles were classed as pathogenic. Each treatment was replicated four times. The data were subjected to ANOVA, and the means were separated by the LSD test.

Results

Survey of carrot plantings

Carrot plantings 6 to 8 weeks age with damping-off symptoms were found in six of the eight regions surveyed. The highest incidence, (25%) occurred in March 1999 (Fig. 3). *A. radicina* was the most common fungus isolated from damped-off seedlings. The pooled monthly data from the eight properties surveyed showed that the incidence of *A. radicina* was highest between January and April 1999.

Distribution of poor establishment

In the Barossa Valley where a planting was intensively surveyed, low seedling densities of 0-10 plants/1m row length were most often found in areas close to irrigation sprinklers. These areas often measured 10 to 30 m in length and 10 to 15m wide and were separated by 5 to 10m. In the cultivar "Thor", 47% of seedlings in the low density regions were infected with *A. radicina*. In the adjacent plantings of variety "Havana", 41% of seedlings were diseased. In the high density seedling areas *A. radicina* was not isolated from roots or collars, but was detected on <1% of plants sampled.

Surveys of mature or semi-mature plants between April and May 1999, showed that in four out of five plantings more than 70% of plants were unmarketable due to restricted crown damage (Table 1). Also on three of those properties more than 40% of the carrots had severe constrictions around the crown.

Isolation of fungi from commercial carrot seed

Three species of *Alternaria* developed on carrot seed at levels ranging from 0.1- 37%. The species *Alternaria alternata* (Fr.) Keissler was found in all batches of seed at levels that ranged from 2-39%. This species produced dark brown conidia in long chains, ellipsoid with a conical or cylindrical beak, approx. 37 μm long and 13 μm wide. *Alternaria radicina* Meier, Drechsler & Eddy was found in 18 of the 19 samples and at levels that ranged from 0-35% infection (Table 2). *A. radicina* was identified by the brown conidia produced in chains of two to three or solitary, and generally ellipsoid approx. 38 μm long and 19 μm wide at broadest part Ellis, 1971). Other species identified were *A. dauci* found on 0.1 to 0.3% of the

seed. The species produced brown conidia usually solitary and occasionally in chains with a beak up to three times the length of the spore, approx. 80 μm long and 6-10 μm wide.

Seed samples with a high incidence of *Alternaria* spp. infestation generally had lower levels of germination compared to those of low levels of infestation. For example, Chantenay, with a 2% levels of *A. radicina* infestation had 85% germination, while Danvers had a 24% level of infestation and 46% germination.

In a separate commercial seed sample in which 16 batches had previously been treated with the fungicides, thiram and/or iprodione, *A. radicina* developed on 11 batches of the fungicide treated seed at levels that ranged between 0.2 to 14% infection. (Table 3).

Pathogenicity tests

All isolates of *A. radicina* recovered from field grown carrot seedlings were pathogenic, but they varied in virulence when tested with two different methods. For example, where carrot discs were used to test pathogenicity isolates of *A. radicina*, those isolates from roots were more virulent than those from petioles and seed (Table 4). By way of contrast in the same tests, isolates of *A. dauci* and *A. alternata* were not pathogenic to seedlings or carrot discs (Table 4).

A. radicina isolates obtained from seedling roots in the SE at Kybybolite, caused severe necrosis to 6 to 8 week old seedlings (Mean disease score = 4), while other isolates from roots and petioles in the Riverland at Paringa were mildly pathogenic (Mean disease scores = 1.2 and 1 respectively). The two isolates of *A. radicina* from Australian carrot seed were also mildly pathogenic to seedlings and carrot discs (Mean disease scores = 2).

DISCUSSION

This survey showed that *A. radicina* was associated with seedling damping-off of carrot seedlings and was widespread in carrot plantings in SA. Damping-off was most frequent in summer and autumn and often developed after conditions of high temperature and heavy rainfalls were recorded. For example at Paringa in 2000, *A. radicina* outbreaks were recorded approximately 30 days after average rainfalls/month and mean monthly temperatures were 1.7mm with 29°C in December and 4.9mm with 34.9°C in February. The distribution of *A. radicina* in a crop in the Barossa Valley showed the disease was not randomly distributed but aggregated.

The different methods of assay for pathogenicity of the strains of *Alternaria* species may reflect the sensitivity of the seedling and carrot disc methods used. These test could also be affected by removal of the protective epidermal cellular layer and subsequent infection of exposed carrot tissue. Another factor affecting variable pathogenicity results is that some carrot varieties have been shown to have resistance to *Alternaria* infection (Pryor et al, 2000).

The high incidence of *A. radicina* on imported and Australian carrot seed showed that the pathogen is common in many of the carrot seed producing areas of the world. The incidence of *A. radicina* was not confined to any particular line of carrot seed as it was detected in 7 different cultivars produced in Australia. The presence of *A. radicina* on many of the seed samples tested suggests that *A. radicina* has been introduced into carrot growing areas in SA by planting infected seed. An example of this was the recent report of damping-off on a property in the Paringa area of the Riverland. Damping-off had not been detected in carrots in this region until recently when a batch of carrot seed cultivar: “Red Hot”, nee “Apache” imported from the USA were sown. The seed was not treated with fungicide and, tests showed that 35% of the seed was infected with *A. radicina*. The development of damping-off was rapid which led the grower to re-seed to compensate for poor establishment. Carrots that survived the early infection by *A. radicina* frequently developed a black ring of decay around the top of the stem, which reduced carrot quality. The fungus also caused petiole infection and rotted stem tissue which usually broke during mechanical harvesting. Similar damping-off and harvesting losses caused by *A. radicina* have been recorded overseas (Pryor et al., 1998).

During seed assays for *A. radicina* healthy seeds adjacent to diseased seeds often became infected during the 21 days incubation at room temperature. Infected seed developed sporulating mycelial strands that grew over the sterile filter paper to infect adjacent healthy seed. This suggests that where seeds are densely planted in soil healthy seedlings may become infected as a result of mycelial spread from infected seed. Seeding rates used in SA are approximately 2 million seeds/hectare. In some of the samples that we tested, 35% of the seed was infested with *A. radicina* and planting this seed would introduce approximately 0.7 million infested seeds/hectare.

Although fungicides had often been applied to carrot seed, our tests showed that they did not always inhibit the development of *A. radicina*. Similar work in New Zealand showed that *A. radicina* was not controlled by treating seed with fungicides (Soteros, 1979b). This contrasts to findings by Maude (1966) who demonstrated complete control of *A. radicina* in seed by soaking for 24 hours at 30°C in 0.2% suspension of thiram (Tetramethylthiuram

disulphide). Pryor (1994) achieved a high level of *A. radicina* control in carrot seed by both hot water (50°C for 20 min) and hot sodium hypochlorite (1.0% at 50°C for 20 min). Further work needs to be done to develop treatments that eradicate *Alternaria* from infected seed. This is particularly important in SA where carrots are planted in new areas previously used for grazing and cereal production.

Acknowledgements

We thank the Horticulture and Research Development Corporation HRDC and carrot growers of South Australia for providing funds to carry out the studies.

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Table 1. The incidence of crown constriction of maturing carrots in three carrot growing regions of SA, April to May 2000

Location	Total sampled	Healthy (0-1) ¹ Sum of ratings	Diseased and unmarketable (2-4) ¹ Sum of ratings
Virginia 1	96	23	73
Virginia 2*	107	9	98
Nuriootpa 1	80	24	56
Nuriootpa 2*	107	61	46
Blanchetown	100	21	79

¹Disease rating 0,1 = Healthy, 2,3,4 = Diseased and unmarketable

* A chi squared test showed that Virginia 2 and Nuriootpa 2 had the biggest influence in the overall chi square

Table 2. Incidence of *Alternaria* spp. on locally produced non-fungicide treated seed and % germination

Cultivar	% <i>Alternaria alternata</i> ^a	% <i>Alternaria radicina</i> ^a	% <i>Alternaria dauci</i> ^a	% Germination ^b
Chantenay	2	2	0	85
Chantenay	34	15	0	74
Nantes	7	3	0	85
Nantes	13	2	0	68
Kuroda	13	19	0	76
Kuroda	11	0	0	75
Kuroda	12	2	0	74
Kuroda	15	4	0.3	82
Kuroda	27	5	0	72
Kuroda	31	6	0	86
Kuroda	37	16	0	66
Flakee	22	5	0	80
Flakee	37	14	0	60
Danvers	36	9	0	71
Danvers	35	24	0	46
Imperator	29	18	0.1	59
Imperator	12	35	0	74
Imperator	39	16	0	96
Amsterdam	26	5	0	85

^a Determined on 500 seeds per cultivar ^b Determined on 200 seeds per cultivar

Table 3. Percent of *Alternaria radicina* from imported and Australian commercial carrot seed treated with both fungicides (Thiram and Iprodione) and % germination

Country of origin	Cultivar	% <i>A. radicina</i> ^a	% Germination ^b
Japan	Mojo	0	>80
Holland	Baby Carrot	0	90
Holland	Flame	0	>80
France	Flame	0	>80
France	Carrisma	0	>80
France	Ricardo	0.2	>80
France	Carrisma	0.4	>80
France	Corona	0.7	>80
France	Ricardo	0.8	>80
Australia	Round	3	81
Australia	Top Weight	4	96
Australia	Uncle	9	52
Australia	Ella	11	91
Australia	Aunt	12	>80
Australia	Kuroda	14	>80
Australia	Flakee	14	60

^aDetermined on 500 seeds per cultivar

^bDetermined on 200 seeds per cultivar

Table 4. Pathogenicity of *Alternaria radicina*, *A. dauci* and *A. alternata* isolates on carrot seedlings and carrot discs, 0-4 scale¹

Fungus and origin	Location	Mean disease score on carrot seedlings	Mean disease score on carrot discs
<i>Alternaria radicina</i> (roots)	Kybybolite	4.0a	2.49c
" " "	Nuriootpa	4.0a	2.5c
" " (leaves)	Nuriootpa	3.75a	3.95a
" " (roots)	Parilla	3.0b	3.42c
" " "	Paringa	2.63c	2.5b
" " "	Paringa	2.54c	1.58d
" " "	Virginia	2.0d	2.0d
" " (seed)	Australia	2.0d	2.0d
" " "	Australia	2.0d	2.0d
" " (roots)	Paringa	1.29e	1.16e
" " (petiole)	Paringa	1.0e	1.21e
<i>Alternaria dauci</i> (leaves)	Paringa	0.13a	0.16a
" " (seed)	Australia	0.13a	0.08a
<i>Alternaria alternata</i> (seed)	Australia	0.08*	0.08*

¹Mean disease scores were based on the numerical means of pathogen symptoms: 0 = no discolouration of tissue, 1 = slight discolouration, 2 = slight discolouration with mycelial growth, 3 =

grey to black necrosis with some production of conidia, and 4 = grey to black necrosis with abundant production of conidia. Means followed by the same letter were not significantly different according to LSD means separation test ($P = 0.05$).

* The pathogenicity of *A. dauci* and *A. alternata* had no significant difference for all locations and no comparison was made because of single sample.

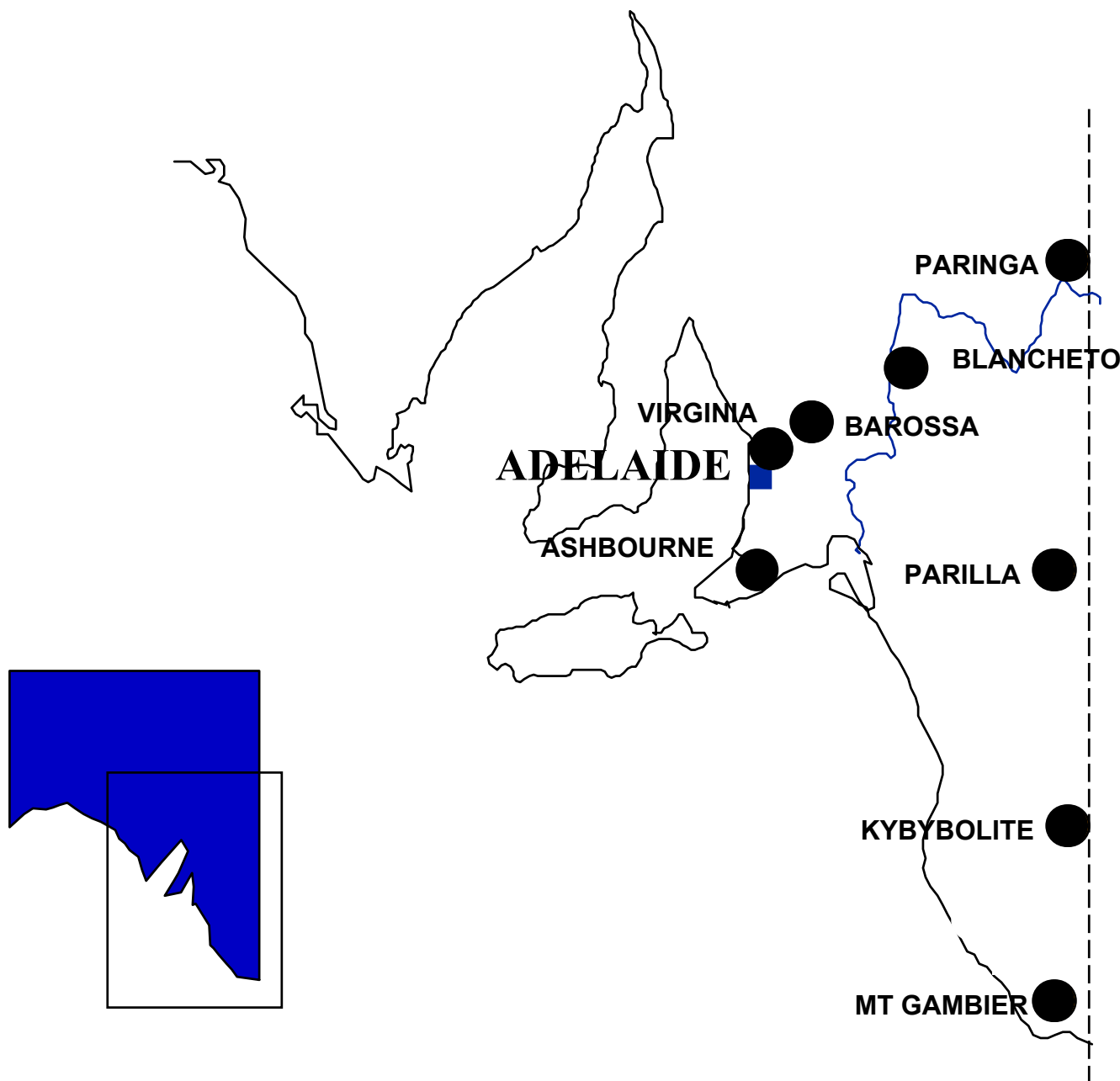


Figure 1. Carrot growing areas in South Australia, 1999

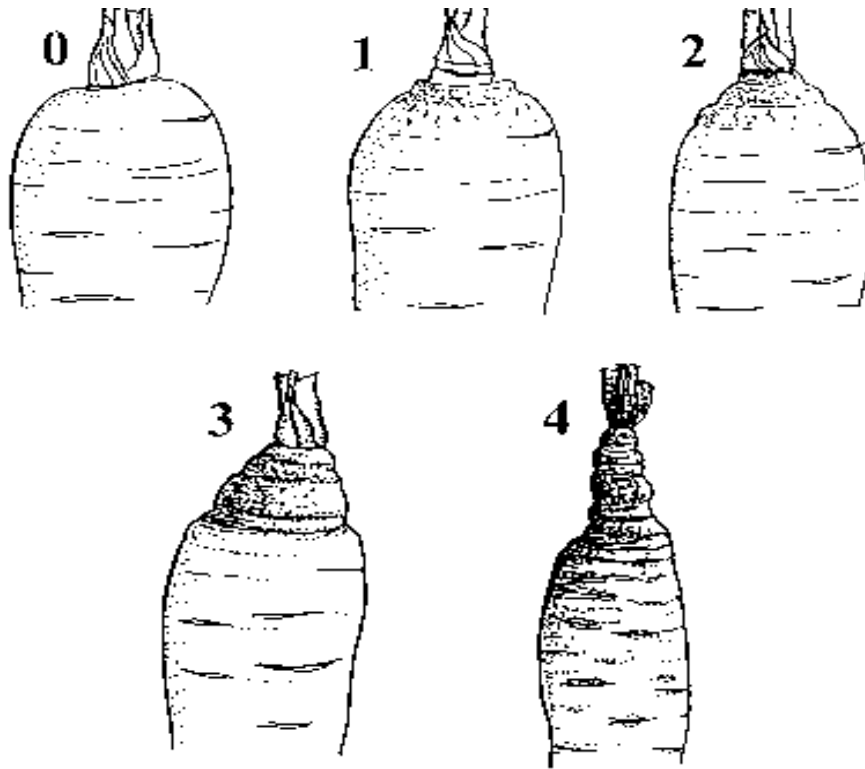


Figure 2. Disease rating for *Alternaria radicina* on collar and shoulder of maturing carrots: 0 = no disease, 1 = slight disease, 2 = moderate disease, 3 = high disease and 4 = severe disease.

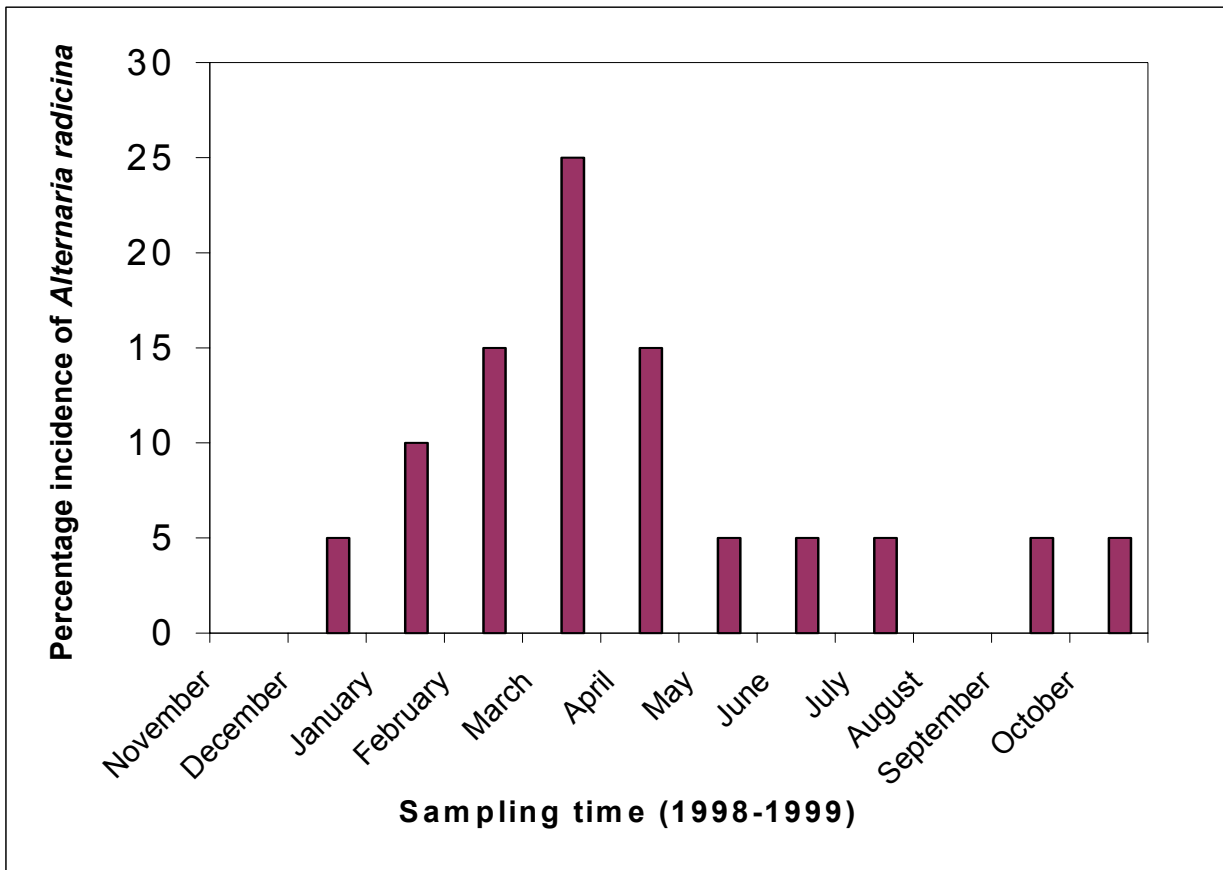


Figure 3. Incidence of *Alternaria radicina* on carrot seedlings in South Australia, 1998-99

Carrot News

Issue 1

February 2001

This is the first in a series of Newsletters produced to provide information on the research into:

CAUSES OF CARROT SEEDLING LOSSES IN SA....

This project is funded by the HAL (Horticulture Australia Ltd) and the Australian Carrot Industry. The work is carried out by the South Australian Research and Development Institute (SARDI) at the Lenswood Horticulture Centre by Robin Coles and Trevor Wicks.

The main aim of the project was to investigate the cause of poor seedling establishment in SA.

Research into the cause of carrot seedling damping-off has been in progress for over 24 months. Some of the main findings of this work are as follows:

What is the extent and the causes of the problem....

- Damping-off is widespread and was found in eight of nine carrot growing areas surveyed throughout South Australia.



Figure 1. Seedling losses caused by *Alternaria radicina*

- The disease is caused by the fungus *Alternaria radicina* and is worst in plantings sampled between January to May.

- The fungus causes damping-off of young carrot seedlings, neck narrowing and crown damage.



Figure 2. Seedling damping off caused by *Alternaria radicina*

- The fungus is common on carrot seed and in soil. Up to 35% of some seed lots are infected. The fungus was found in seed treated with fungicides and research is continuing to develop more effective seed treatments.

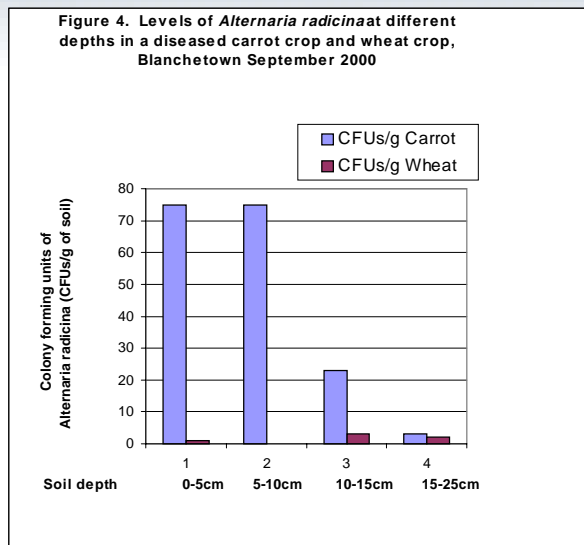


Figure 3. Germinating carrot seed infected with *Alternaria radicina*



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- *Alternaria radicina* survives in soil for more than 8 years. Highest levels are in the top 15cm of soil (See Fig.4. below).



- The fungus infects semi-mature and mature plants causing a constriction around the crown.



Figure 5. Crown constriction caused by *Alternaria radicina*

Future issues will include results of:

- Seed treatments
- Testing other pathogenic fungi
- Fungicide screening

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CARROT NEMATODE SURVEY

By Greg Walker

This project started in September 2000 and is part of a collaborative project funded by the HRDC, titled "Improved Control of Nematodes in Carrot Production"

- A survey of carrot crops is being undertaken nationally to assess the distribution and abundance of nematodes, and their effects on carrot productivity
- Preliminary results show that nematodes are more frequently a problem where short rotations are used and/or chemical control is absent
- Organic properties are particularly susceptible to development of nematode problems
- Nematodes collected are being identified. *Meloidogyne javanica* is so far the most commonly occurring species of Root Knot Nematode detected in South Australian carrots.
- A range of other parasitic nematodes has been detected
- Field trials have been established to correlate nematode levels with yields
- Soil/plant samples are required at planting and at harvest, samples can be sent to me at the address listed below or I can be contacted on 08 8303 9355 for further information.

We wish to thank HAL and carrot growers for their assistance in this work.



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Carrot News

Issue 2

July 2001

This is the second in a series of Newsletters produced to provide information on the research conducted in SA:

CAUSES OF CARROT SEEDLING LOSSES IN SA....

This project started December 1998 and is funded by HAL and the Australian Carrot Industry. The work is carried out in the South Australian Research and Development Institute (SARDI) by Robin Coles and Trevor Wicks.

The main aim of the project was to investigate the cause of poor seedling establishment in SA and formulate management strategies.

Research into the cause of carrot seedling disease has been in progress for over 30 months. Some of the findings are as follows:

Carrot seed contains fungi that cause seedling losses....

- *Alternaria* species that cause disease enter the immature seed coat at the time of carrot flowering.



Figure 1. Carrot flower seed crop with *Alternaria* infection.

- The fungus survives for up to 8 years in seed with levels increasing the longer seed is kept in storage.
- *Alternaria radicina*, is the main fungus causing seedling damping-off.



Figure 2. Close up of carrot flower with *Alternaria* infection on floral parts.

- The fungus was found in most imported and some Australia carrot seed. Levels up to 35% were found in some seed lots.
- *Alternaria radicina* was also found in many fungicide treated seed lots, with several having disease levels as high as 14% in some samples.
- *Alternaria radicina* has been introduced into most carrot growing soils by contaminated seed.

How to control seed-borne *Alternaria* diseases...

- Seed soaked in hot water at 50°C for 30 minutes completely controlled seed-borne *Alternaria* with only a 4% loss in germination.
- Seed soaked in a 1% solution of Potassium permanganate at 22°C reduced seed borne disease by 75%, with only a 4% loss in germination.
- Seed treated with steam at 51°C for 30 minutes completely controlled disease but reduced germination by 11%.



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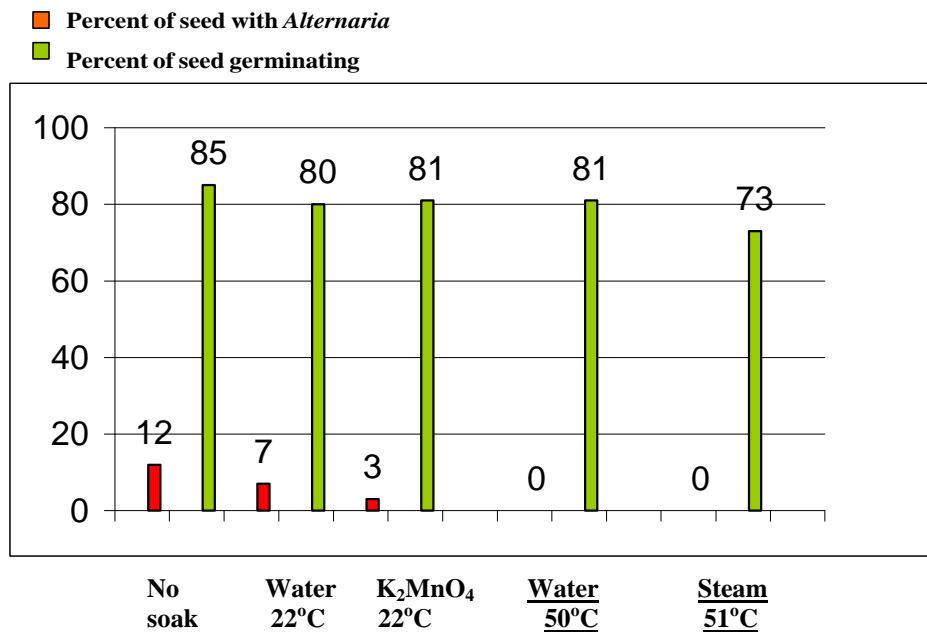


Figure 3. Carrot seed treatment, percentage *Alternaria* survival and percentage seed germination

SURVEYING CARROTS FOR CARROT VIRUS Y

As part of a HAL funded collaborative national survey with Agriculture Western Australia, carrot growing areas are being surveyed to determine the incidence of Carrot Virus Y in SA.

Carrot virus Y and its impact in SA.

- Carrot virus Y (CVY) is spread by aphids and causes severe distortion and knobbliness of the mature carrot.
- Plants that are infected 4 weeks after sowing develop severe root distortions.
- Carrots infected with CVY have been found in most states and were first recorded in SA during 1972.
- In WA, CVY was first noted in 1997 with an infection incidence of 65% at one property.
- The symptoms are most common in spring harvested carrots, with stunted shoot growth, leaflets yellowing and having a feathery appearance.
- So far the only known host plant for the virus is carrots.
- CVY has been detected in the limited number of samples taken in SA in 2001.



Figure 4. Carrot with carrot virus Y on right and a healthy carrot of the same age on the left.

Future issues will include results of:

- Fungicide trials at Virginia
- Recently found pathogens of carrots

We wish to thank HAL and carrot growers for their assistance in this work.

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Carrot News

Issue 3

February 2002

This is the third in a series of Newsletters produced to provide information on the research conducted in SA:

MANAGING ALTERNARIA BLIGHT IN CARROTS....



This project started July 2000 and is funded by HAL and the Australian Carrot Industry. The work is carried out in the South Australian Research and Development Institute (SARDI) by Robin Coles and Trevor Wicks.

The main aim of the project was to investigate the cause of poor seedling establishment in SA and formulate management strategies.

Research over the past 36 months has shown that the seed and soil borne fungus *Alternaria* as the main cause of the problem. Trials to investigate effectiveness of fungicides applied to seedlings has been initiated.

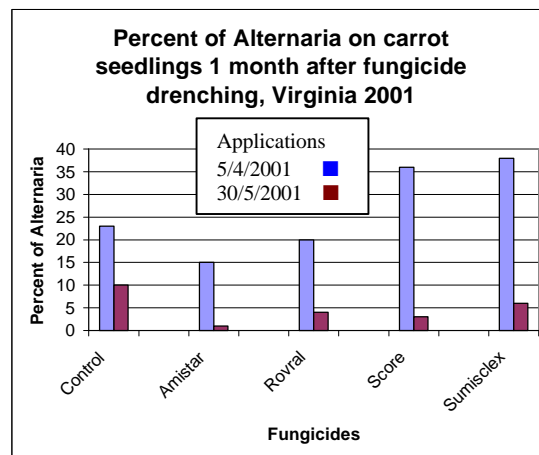
Fungicide trials at Virginia....

- Four fungicides were trialed on 6 and 8 week old carrot seedlings at Virginia.
- In two separate experiments the fungicides: Amistar, Rovral, Score and Sumisclex were applied as water drenches (Fig 1).
- The fungicides were applied with a watering can to simulate sprinkler application in April and May 2001 when plants were at the two to three leaf stage.
- Seedlings were assessed for the presence of *Alternaria* one month after the fungicides were applied.
- In both experiments, Amistar and Rovral reduced the percentage of *Alternaria* infections on fungicide treated seedlings assessed one month after application (Fig 2).
- Score was more effective when applied to 8 week old seedlings (Fig 2).

Figure 1: Fungicides being applied as seedling drenches



Figure 2: Percent of *Alternaria* on carrot seedlings 1 month after applying fungicide drenches



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New pathogens of carrots in SA:

Closer examination of fungi isolated from carrots in SA has identified two new diseases.

Alternaria radicina var *carotiincultae*

- *Alternaria radicina* var *carotiincultae* was isolated from harvest and storage carrots. In trials on potted seedlings it caused rapid decay of both roots and leaf tissue.
- The pathogen was found in two carrot growing areas near the eastern border of SA.

Figure 3: Locations in SA where *Alternaria carotiincultae* has been recorded

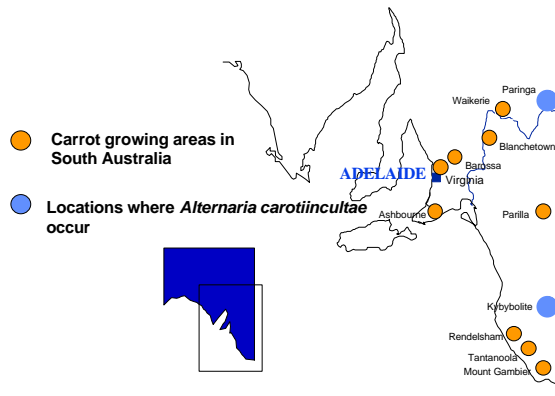
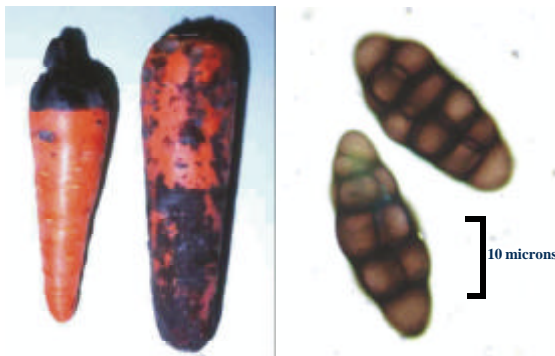


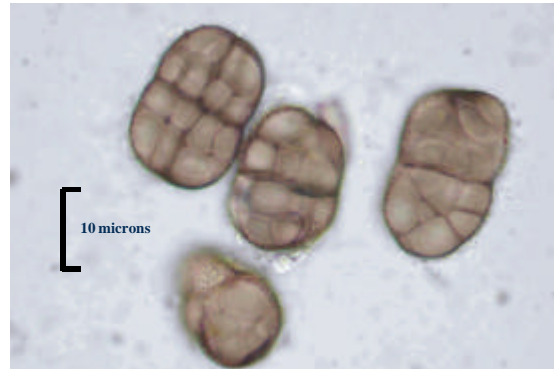
Figure 4: *Alternaria carotiincultae* carrot rot on cold storage carrots on the left and conidia on the right



Stemphylium botryosum

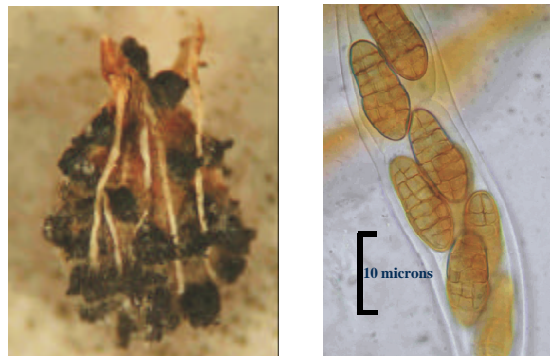
- *Stemphylium botryosum* and its sexual stage *Pleospora herbarum* affects carrot seed and leaf tissue. This fungus produces browning of the older leaf tips but is a moderate pathogen of carrots.

Figure 5: Conidia of *Stemphylium botryosum*



- *Pleospora herbarum* is the sexual state of *S. botryosum* and occurs on carrot seeds

Figure 6: Carrot seed with *Pleospora herbarum* on the left and ascospores on the right



Future issues will include the results of:

- Fungicide seed treatments
- Fumigation treatments and *Alternaria*

We wish to thank HAL and carrot growers for their assistance in this work.

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Carrot News

Issue 4

August 2003

This is the fourth in a series of Newsletters produced to provide information on the research conducted in SA:

CARROT VIRUS Y UPDATE IN SOUTH AUSTRALIA

Horticulture Australia Limited has approved research funding to study the management of Carrot Virus Y in the Virginia area of SA. \$50,000 has been provided via Agriculture WA to develop a management plan. The virus was first detected in Virginia in the 1970s and causes severe distortion of the roots i.e. "Michelin carrots". High levels were detected throughout many of the carrot plantings during January to March this year, with some crops being unmarketable, Figure 1.

Infected plants' leaves are mottled and yellow, with reddening and dying off near the edges. They take on a feathery appearance (Figure 2), and plants are sometimes stunted. Carrots from plants infected early are stubby and show severe distortion and knobliness. Plants infected latter are thinner and longer than early infected ones, and develop mild distortions.

Aphids spread carrot virus Y, and when aphid numbers are high (particularly in the spring and autumn) crops are most likely at risk of high infection levels. When carrots are infected early (up to six weeks after seeding) symptoms are severe. Aphids transmit carrot virus Y from adjacent infected carrot crops to newly sown ones.

A steering committee will oversee the project. The project will involve trapping aphids in Virginia during spring 2003 to summer 2004 to determine aphid species, numbers and flights patterns. A search for alternative host plants acting as a reservoir for the virus will be undertaken, and recommendations for planting times and aphid control completed by February 2004. An integrated disease management strategy for the control of carrot virus y will be released nationally by Agriculture Western Australia in September 2004.

The appointed members to the steering committee are Figaro Natoli, (Carrot Association for Research and Development WA Inc.), Barry Nicol (Grower and committee advisory member to HAL for root

vegetables), Lindrea Latham (virologist, Department of Agriculture, Western Australia), Dr Nancy Schellborn (SARDI entomologist), Craig Feutrill (SA vegetable industry development officer) and Robin Coles (SARDI Research Officer).

Figure 1: Harvested carrots with severe distortion caused by Carrot virus Y - "Michelin Carrots".



Figure 2: Carrot Leaves. Left: Healthy. Right: Carrot virus Y.



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Carrot Seed Research Update

This project is funded by Horticulture Australia Limited (HAL) and the Vegetable Industry. Commenced in 1999 and due to finish in September 2003. The final report is due in October 2003 and will be available from HAL.

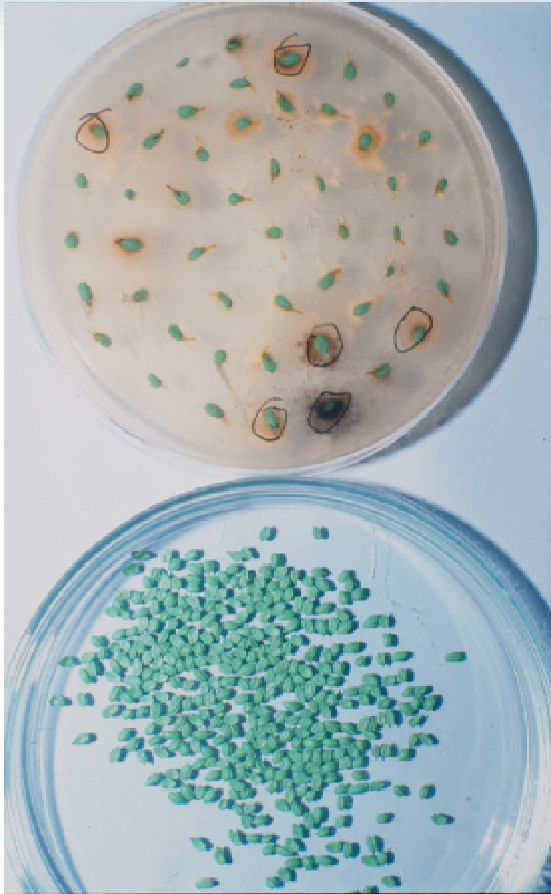
Recently a commercial carrot seed batch coated with fungicide was detected with a 2% level of *Alternaria*. The introduction of this fungal disease into new ground can cause seedling damping-off and pack out and cold storage losses.

When planted at a rate of approx. 1.2 million per seeds hectare, 2% infection results in an introduction of twenty four thousand point sources of *Alternaria* per hectare. Overseas research has shown that twenty colonies of *Alternaria* in a gram of soil can cause a disease outbreak. Use of infested seed has contaminated the soil for future carrot production in some areas.

In addition, the *Alternaria* strains isolated from the seed were tolerant to Rovral (Iprodione). Tolerance to iprodione has been found in SA, but is not widespread. This common fungicide is used as a seed coating and concerns have been raised that it may no longer be effective in controlling all seed-borne fungal infestations. Tests are currently underway in SARDI to determine if iprodione is effective in controlling some *Alternaria* infections on carrot plants when applied as a foliar spray.

SARDI offers a seed testing service for *Alternaria* levels in carrot seed, based on 500 seeds per lot, with an estimated diagnostic period of fourteen days. A soil test is also available to determine levels of *Alternaria* in the soil. These tests and all others undertaken by the Horticultural Diagnostic Service are listed on the web site (<http://www.sardi.sa.gov.au/horticulture/index.html>).

Figure 3: Top: Alternaria radicina growing from fungicide coated carrot seed in a selective media. Bottom: Fungicide coated seed.



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Future issues will include the results of:

- Aphid monitoring at Virginia
- Carrot virus Y incidence in Virginia

We wish to thank HAL and carrot growers for their assistance in this work.

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Carrot fungus in hot water

A SEED fungus which can rob carrot growers of up to a quarter of their crop is being successfully tackled by a soak in a good hot bath.

SARDI scientists have found that the internationally troublesome *Alternaria* fungus, can be killed by giving seeds a 10 to 20 minute dip in water heated to around 50 degrees celsius.

The fungus principally stifles germination, but can also render mature plants and carrots useless.

Research officer Robin Coles said the fungus had also proved to be vulnerable to a combination of hot air and steam during tests at SARDI's Lenswood Centre.

"We also tried a range of fungicides on carrot flowers and found several very effective in eliminating *alternaria* during seed set," Mr Coles said.

The oxidising agent potassium permanganate was also very effective in controlling fungal diseases. Hydrogen peroxide treatment also had shown positive results.

Alternaria is a problem for carrot growers around the world and is thought to have entered Australia via seed imports from the northern hemisphere.

The fungal pest can reduce on-farm production by up to 25pc, can live in soil for up to 11 years and can pose a risk to the industry's export reputation as carrots may develop *alternaria* infection in cold storage.

"Even juicing carrots, which are the lowest grade of carrot, can't be used if high levels of *alternaria* are present, so it causes problems for the industry on virtually every level," Mr Coles said.

At a glance

- SARDI is developing treatments to protect the State's \$50 million carrot industry.
- Hot water treatment can combat *Alternaria* fungus
- Fungus can cut crop germination by up to 25pc and survive in soil for up to 11 years.

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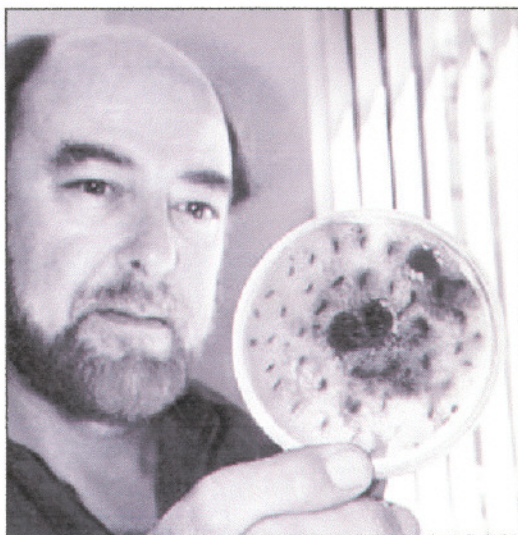
He believes seed soaking may be one of the keys to overcoming the fungus, but that even more effective programs could be based on treating the carrot plant's flower.

"If we can treat the flower, which is where the next generation of seeds comes from, we are really getting at the heart of the problem," he said.

On-farm experiments with flower treatments at the Lenswood Research Centre were already getting excellent results and commercial programs were likely to evolve from these successes.

"A successful campaign against *alternaria* would be a tremendous boon for the carrot industry as a whole and would greatly benefit individual growers," Mr Coles said.

• Contact: Robin Coles 8389 8817.



SARDI's Robin Coles inspects seeds affected by *alternaria*.

SARDI celebrates a decade of excellence

THE South Australian Research and Development Institute has been recognised and praised for its scientific excellence and continuing positive economic and social contributions to South Australia by Agriculture, Food and Fisheries Minister Paul Holloway.

Speaking at SARDI's recent 10-year anniversary celebrations at the institute's Urrbrae headquarters, Mr Holloway told a crowd of more than 200 industry representatives, other invited guests and staff that SARDI is the leading food science body in the state.

It is also one of the national leaders in this field and was increasingly being recognised around the world.

"South Australia's economy has benefited significantly from SARDI's research and the economic flow-on effects it generates through farming, industry and the broader community," Mr Holloway said.

"In its first 10 years, the application of SARDI's technologies and products has generated a predicted \$8 billion in additional gross state product for the South Australian industry. Economic projections predict that benefit to the State

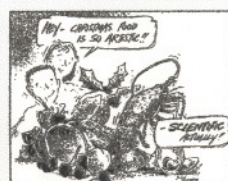


A plaque commemorating SARDI's first ten years of achievement was unveiled by Minister Paul Holloway.

will be worth at least a further \$10b between now and 2020," he said.

The crowd, who gathered for the night in SARDI's spectacular Plant Research Centre greenhouse atrium, also heard speeches from original institute chief executive Dr John Radcliffe and current Executive Director and founding SARDI member Rob Lewis. They both paid tribute to the enthusiasm, intellectual input and hard work of SARDI's staff during the past decade.

Each said the research body could not have had its many successes without a team of people dedicated to their work and to the further development of the industries they serviced.



Hard work behind the hamper

CHRISTMAS is a time for family, friends and plenty of festive foods - and many of these products are being worked on by the South Australian Research and Development Institute for the benefit of producers and consumers.

Here is a scientist's hamper of everyone's favourite seasonal foods and the work going on to improve them.

Chicken and ham are central to the traditional English Christmas dinner and SARDI is working to improve these meats by researching new free range farming systems for pigs and poultry. These are aimed at producing animals with more meat and improved tenderness through reduced stress.

In the modern Australian Christmas tradition, a fresh rock lobster often takes the chook's place as the centrepiece and needs to look as good as it tastes, so SARDI's work in this area may lead to more spectacular table settings.

The rock lobster can be given richer shell colour through managed malting and it will also grow back claws and legs lost in fights when it grows a new shell.

SARDI also does ongoing assessment of rock lobster stocks and by determining the basis for a sustainable catch, it helps ensure a Christmas supply each year.

South Australia's delicious oysters are another seafood delicacy for the festive season. SARDI is working on improving their taste and texture by exploring how harvesting and handling stresses might affect the delicate meat.

For sweets, Australians are lucky to be able to enjoy fresh summer fruit at Christmas time, but traditional dried fare remains popular. SARDI's horticultural scientists are working on developing a sweeter dried apricot through the crossing of a sweet Turkish variety with a flavoursome European fruit.



Mature carrots badly blackened by the fungus.



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