Can crop rotation reduce inoculum of the carrot pathogen *Alternaria radicina*?

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Abstract

The fungal pathogen *Alternaria radicina* causes pre- and post-emergence damping off, leaf and umbel blight and black rot of carrot. It is seed and soil-borne, and in mid-Canterbury, soil populations ranging from 33-223 colony forming units (CFU)/g soil have been recorded. How long *A. radicina* remains viable in New Zealand soils was not known. Fields which had last produced a carrot seed crop from twelve down to one year previously were identified and their *A. radicina* population determined. The pathogen was still present six years after a carrot seed crop had been harvested, but was absent from soils where a carrot seed crop had been harvested seven or more years previously, suggesting that a seven to eight year gap between carrot seed crops in the same field would be required to avoid disease problems from this source. In a pot trial, *A. radicina* inoculum was reduced by 29% (from 240 CFU/g) after four and a half months in the presence of wheat plants, and was also significantly reduced in the presence of barley and faba bean, but not perennial ryegrass or pea. It is possible that root exudates contain antifungal compounds, but this was not determined. Whether growing the non-host crops wheat, barley or faba bean after carrot can reduce *A. radicina* soil-borne inoculum requires further investigation.

Additional keywords: *Daucas carota*, soil-borne inoculum, carrot seed production, root exudates, wheat, barley, faba bean

Introduction

The fungal pathogen *Alternaria radicina* can infect all parts of the carrot plant, both above and below ground, causing pre- and post-emergence damping off, leaf and umbel blight, and black root rot (Pryor, 2002). As a seed-borne pathogen it is believed to have entered New Zealand via imported carrot seed lots (Scott and Wenham, 1973), but it is also soil-borne. In mid-Canterbury, *A. radicina* soil populations ranging from 33 to 233 colony forming units (CFU) /g soil have recently been recorded (Trivedi, 2010). The threshold level for black root rot is around 20 CFU/g soil (Pryor *et al*., 1998). This development of soil-borne inoculum has been associated with the establishment of carrot seed production in mid-Canterbury over the past 20 years (Trivedi, 2010).

While root rot and foliar infections can reduce seed yield, the major problem for New Zealand's carrot seed producers caused by *A. radicina* is the pathogen's effect on reducing carrot seed germination. Fungal attack of the seedling root and/or

shoot tissues may either kill the seedling, or cause necrosis to the extent that under the germination rules of the International Seed Testing Association (ISTA, 2012) infected seedlings are classed as abnormal. Over the past ten years, the failure of some carrot seed lots to meet contracted germination standards because of the effects of *A. radicina* has resulted in their rejection by the European seed houses for which the hybrid carrot seed was being produced (Trivedi, 2010).

Control of soil-borne inoculum of *A. radicina* has been investigated using physical (ploughing) and chemical (soil fumigation, fungicides) methods. While ploughing initially reduced inoculum levels in the 0-20cm soil zone, the effect did not last past the first year (Farrar *et al*., 2004). Fumigation using metam sodium increased (Coles *et al*., 2005) or delayed (Trivedi *et al*., 2011) soil-borne inoculum build up in plot trials, and was not considered practical for broad acre use. Similarly, fungicide soil drenching initially reduced soil-borne inoculum one month after application, but by 32 weeks after application *A. radicina* population density had begun to increase (Trivedi *et al*., 2011). *A. radicina* is a long-lived pathogen in soil, and has been reported to survive for eight years without a host (Farrer *et al*., 2004). Cropping with non-host crops has been proposed as a method for control of the pathogen, with wheat (Coles and Walker, 2001), barley, beans and lucerne (Farrar *et al*., 2004) being suggested as suitable species. For example Coles and Walker (2001) reported that when comparing carrot and wheat as the next crop in *A. radicina* infected soil, CFU/g in the top 10cm of soil was 75 for the carrot but less than 2 for the wheat. This aspect of control of *A. radicina* had not been previously investigated in New Zealand. In this paper the results from a field survey and a glasshouse experiment designed to determine the effect of the succeeding crop on soil-borne *A. radicina* are reported.

Materials and Methods

Field Survey

With the assistance of Midlands Seed Ltd field staff, 12 fields in mid-Canterbury were selected because they had previously grown carrot seed crops known to be infected by *A. radicina*, and the subsequent cropping history following the carrot seed crop was mostly known (Table 1). For example, at Farm 1 carrot seed was harvested in 1995 but not subsequently in that field, while at Farm 12 the carrot seed crop had been harvested in 2006.

The *A. radicina* soil population density was determined for each field. Four small (20 × 20m) plots within each field were selected at random, and from within each plot soil was sampled from 20 different randomly selected places along a zigzag traverse to a depth of 5cm using a 2.5cm diameter soil sampler. This sampling took place during September 2006. Soil samples from each plot were ambient air dried for one week before being crushed using a mechanical grinder and passed through a 850μm sieve (Endecotts Ltd, London). Five grams of this processed soil was then added to 45ml autoclaved water agar (0.2% agar) in a 250ml flask which was shaken on a wrist action shaker (Griffen) at 1000 rpm for 15 minutes. Six 1 ml aliquot replicates were then taken and each spread evenly onto the surface of an *A. radicina* selective agar (ARSA - Pryor *et al*., 1998). After 14 days incubation at 28°C in the dark, the under surface of each plate was examined for the distinctive black colonies of *A.*

radicina growing down into the agar (Trivedi *et al*., 2011). CFU/g soil were then calculated and the data for the four replicate plots meaned to obtain the field soil population density.

Glasshouse Experiment

Silt loam soil was collected from a mid-Canterbury field which had not previously grown carrot (R. Wilson, pers. comm., 2008) and a sample tested for the presence of *A. radicina* using the dilution plate method described in the previous section. The pathogen was not detected. The required amount of soil required to fill each of six sets of ten 4 litre pots (42 kg) was weighed out and each of the six soil lots spread evenly onto a plastic sheet spread on the ground. Five soil lots were then inoculated with a mixed conidial suspension of three isolates of *A. radicina* at a level which delivered 250 CFU/g soil (Trivedi *et al*., 2011). The sixth soil lot was sprayed with sterile water. After hand mixing the soils were placed into the pots and left undisturbed in a glasshouse for 14 days (16-30 March 2008; 12-30^{o}C) to allow the pots to stabilize.

Seeds of each of five non-host crop species likely to be included in a rotation on mid-Canterbury arable farms *viz* barley, wheat, pea, faba bean and perennial ryegrass, were sown on 31 March 2008 into their 10 pots (4 seeds/pot at a depth of 5cm), while one set of pots was left fallow. The resulting plants were left undisturbed in the glasshouse for four months (temperature range 10-25°C) with overhead watering weekly or as required. On 31 July 2008 all plant material was cut at soil level and removed. The soil from each pot was sampled to a depth of 5cm using the previously described soil sampler from three randomly selected positions in each pot. The *A. radicina* soil population density of each pot was then measured using the dilution plate method and ARSA.

Data Analysis

For the field survey, *A. radicina* population densities are presented with the standard error of the mean. As the glasshouse trial data were normally distributed, non-transformed data were analysed using a one-way analysis of variance (ANOVA). Means were tested for significance using least significant difference (LSD) (P=0.05).

Results

Field Survey

A. radicina was not detected in soils from fields where a carrot seed crop had been harvested between 1995 and 2000 (Figure 1). However, the pathogen was present in soils from fields where a carrot seed crop had been harvested from 2001 to 2006. There was variation in CFU/g soil among years, but the highest population (133 CFU/g soil) was found in soil from the most recently harvested field surveyed (Figure 1).

Glasshouse Experiment

In the fallow pots, *A. radicina* density four and a half months after soil inoculation was 240 CFU/g soil (Figure 2). Soils in which wheat, barley and faba bean were grown had a lower ($P< 0.05$) *A. radicina* population, but those in which perennial ryegrass and pea were grown did not differ from the fallow control (Figure 2).

Table 1: Succeeding crops grown in the same field after a carrot seed crop on twelve mid-Canterbury farms.

Years of carrot seed harvest	Farms											
	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8	Farm 9	Farm 10	Farm 11	Farm 12
1995	Carrot											
1996	-[1]	Carrot										
1997	-	-	Carrot									
1998	-	-	Process pea	Carrot								
1999	Wheat	Process pea	Wheat	Maize	Carrot							
2000	Radish	Grass seed	Turnip/Dry Pea	Turnip/Maize	Fallow/Maize	Carrot						
2001	Wheat/ Turnip	Pasture	Barley	Process pea	Fallow/Maize	Fallow/Maize	Carrot					
2002	Process pea	Maize	Grass seed	Grass seed	Wheat	Oats/Barley	Oats/Maize	Carrot				
2003	Triticale	Oats/ Maize	Pasture	Pasture	Cabbage/Radish	Grass seed	Oats/Maize	Pasture	Carrot			
2004	Grass seed	Oats/ Process pea	Maize	Maize	Pea	Pasture	Wheat/Oats	Pasture	Oats/Maize	Carrot		
2005	Pasture	Radish	Process pea	Broad bean/ Broccoli	Grass seed	Pasture	Process pea	Process pea	Oats/Maize	Oats/Maize	Carrot	
2006	Process pea	Wheat	Grass seed	Wheat	Pasture	Chinese cabbage	Grass seed	Red beet	Broccoli/Squash	Wheat/Oats	Oats/Squash	Carrot

[1]Records are not available.

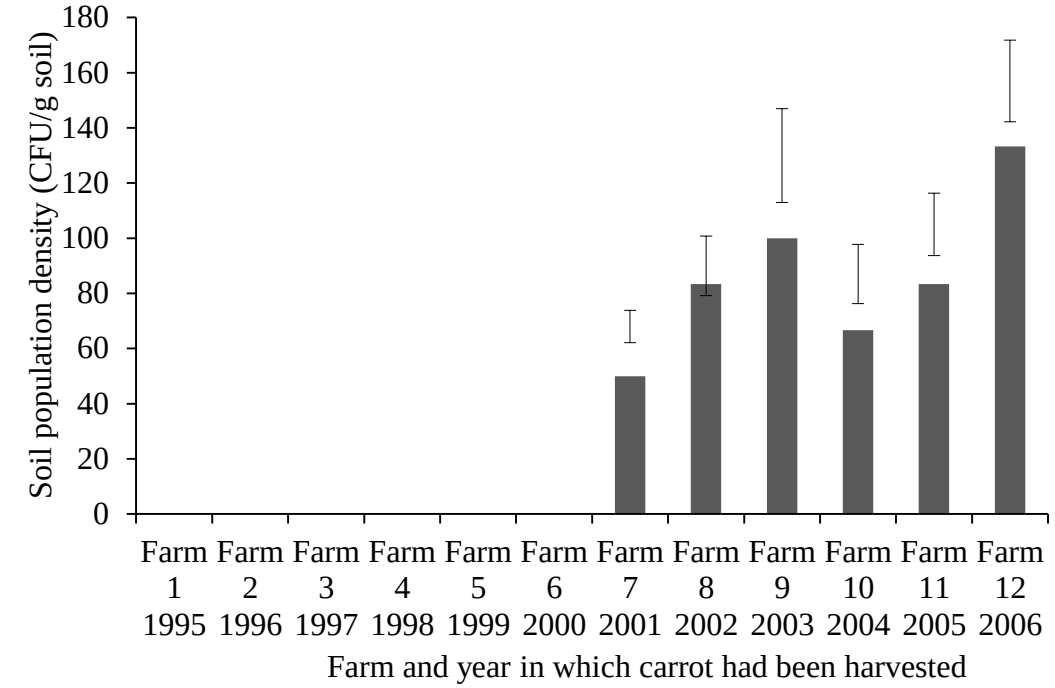

Figure 1: Soil population density of *Alternaria radicina* in fields on mid-Canterbury farms where a carrot crop had been harvested in different years. The bar indicates standard error of the mean.

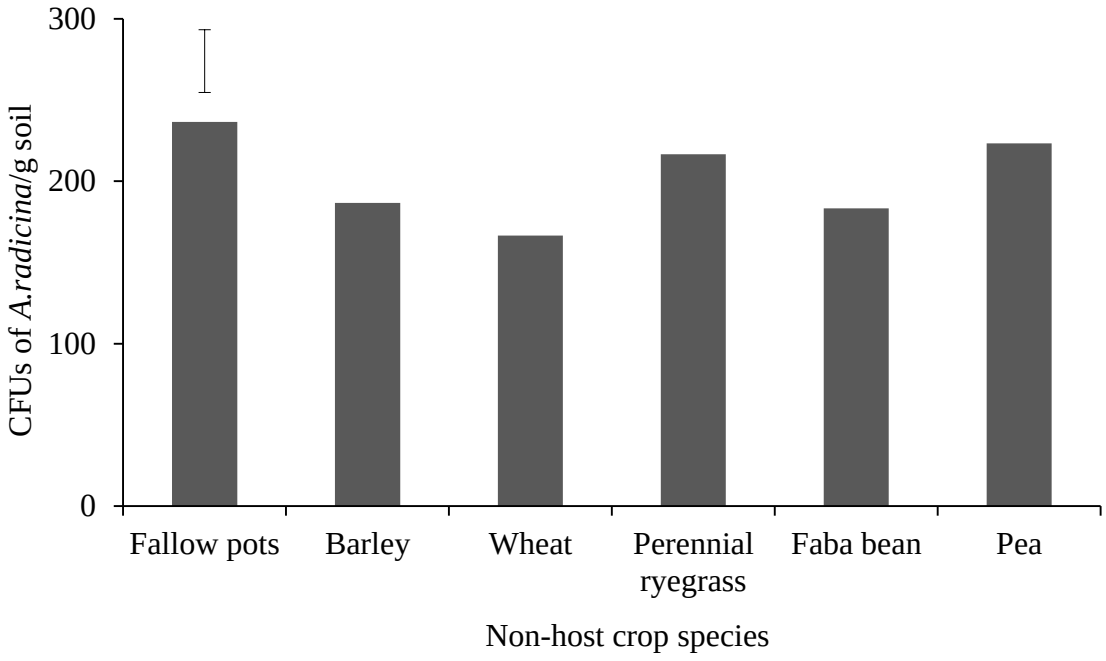

Figure 2: The effect of different crops on the soil population density of *A. radicina*. The bar indicates Fisher protected $LSD_{(0.05)}$.

Discussion

The field survey results suggest that in mid-Canterbury, at least seven years without a carrot crop was required to eliminate/minimise *A. radicina* soil-borne inoculum. In the United Kingdom Maude and Shuring (1972) suggested that at least eight years were required to manage soil-borne inoculum in heavily infected fields, while Pryor *et al.* (1998) found that the pathogen could survive in dry soils for four years. Conidia are the survival propogules (Pryor *et al*., 1998; Trivedi, 2010) and while carrot plant debris in soil will aid survival, the fungus can also survive readily in the absence of carrot plant debris. Trivedi (2010) showed that *A. radicina* recovery from soil two years after inoculation was 70% in the absence of carrot plant debris compared with 80% in the presence of debris.

What cannot be determined from the survey is whether the presence or absence of the pathogen was entirely a factor of time since a carrot crop was last grown, or whether any of the succeeding arable crops also had an effect. Did the inclusion of wheat in the rotation for Farm 10 allow a greater reduction in *A. radicina* inoculum than that which occurred at Farm 9 where wheat was not grown? Or does the recorded difference in CFU/g soil (75 compared with 100) simply reflect different initial inoculum levels? It is tempting to speculate that wheat was a factor, considering the results reported by Pryor *et al*. (1998) who showed that in five Californian fields sampled immediately after the carrot harvest, then resampled in each of the next two years following wheat crops, *A. radicina* populations had decreased by an average of 37% and 79% respectively, a result also supported in another study by Coles and Walker (2001) in South Australia.

In the glasshouse experiment, the presence of wheat had reduced the *A. radicina* soil population by 29% after four and a half months, and barley and faba bean had also significantly reduced the inoculum within this short time frame. Farrar *et al*. (2004) recommended sowing wheat, barley, bean and lucerne after carrots as one management tool to reduce *A. radicina* soil-borne inoculum.

The differential responses of plants to pathogenic micro-organisms are not as yet well understood (Doornbos *et al*., 2012). Plant roots exude an enormous range of small molecular weight compounds into the rhizosphere (Bais *et al*., 2006) and those exudates determine the population of rhizosphere microorganisms (Rovira, 1969). Specific root exudates can affect soil-borne fungal pathogens in two ways: they may be antifungal substances which directly kill/inhibit the pathogen (Park *et al*., 2004), or they stimulate the growth of an antagonist in the rhizosphere which competes with the pathogens (Bais *et al*., 2006).

There are some reports that species used in the glasshouse experiment can either produce, or induce the production of, antifungal compounds. For example, wheat is known to encourage the growth of a fluorescent *Pseudomonas* species which produce an antifungal compound (Mazzola *et al*., 2004), and Tang *et al*. (1975) reported the production of an antifungal compound by wheat roots. Park *et al*. (2004) demonstrated that these products, which are also produced by maize roots, inhibit the growth of *Fusarium oxysporum*. Barley roots also produce antimicrobial exudates against *F. oxysporum*, but only in the presence of an arbuscular mycorrhizal species (Steinkellner *et al*., 2008). Fawcett *et al*. (1969) reported that broad bean

exudates contained a wyerone substance which was toxic to many fungi including *A. brassisicola*. Pea root exudates can also contain antifungal compounds, but antifungal activity of perennial ryegrass exudates has not been reported (Trivedi, 2010). Whether any of the compounds exuded from wheat, barley or faba bean roots have any direct effect on *A. radicina* is yet to be determined. If activity is confirmed, then the elucidation of the active ingredient(s) may allow an understanding of the control of this pathogen via crop rotation, and/or provide an interesting possibility for biocontrol.

Conclusions

A. radicina can still be present in field soil six years after a carrot seed crop had been harvested but absent from soils where carrot seed had been harvested seven or more years previously, suggesting that a seven to eight year gap between carrot seed crops in the same field is necessary to avoid disease problems arising from soil-borne inoculum.

Levels of *A. radicina* soil-borne inoculum may be reduced more quickly by growing wheat, barley or faba bean after the carrot seed crop, probably because of the abilities of these non-host species to produce antifungal root exudates antagonistic to *A. radicina*, but whether this could become an effective control mechanism requires further investigation.

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