Thermal control of disease in carrot seed crops

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Abstract

Alternaria radicina is a soil- and seed-borne fungal pathogen of carrot, which initially causes an infection on the lower leaves/petioles and eventually black rot of the carrot root. The effects of thermal treatments (flaming and steaming) were first investigated in commercial carrot seed fields in Canterbury, New Zealand in September 2006. A tractor mounted burner and a steamer were driven at 1.7, 2.3 and 2.7 km h^{-1} over carrot plants, destroying the infected carrot foliage. Plants regrew to normal size within 60 days, with no effect on their overall dry matter accumulation. Foliage infection was initially reduced by the treatments, but by three months after treatment, disease incidence was similar to that of the control. Steaming significantly reduced ($P \leq 0.05$) black rot disease ratings on carrot roots but flaming did not. Steaming was investigated again in the following season, using the same speeds but with two different application dates (5 June and 20 July 2007). Treated plants took 80 days to recover their growth, and again there were no adverse effects on overall dry matter accumulated after this period. As before, foliage disease ratings were initially reduced but were similar to the untreated control by 3-4 months after treatment. Steaming at 2.3 km h^{-1} and 2.9 km h^{-1} in the first week of June gave better control of black rot ($P \leq 0.05$) than all other treatments but did not reduce carrot seed infection by *A. radicina*, which probably resulted from wind borne inoculum from surrounding external sources.

Additional keywords: *Alternaria radicina*, flaming, steaming, carrot seed, black rot, *Daucus carota*

Introduction

About 50% of the world's carrot (*Daucus carota* L.) seed is grown in Canterbury, New Zealand (Keast, 2006). Carrot is a biennial crop and requires 14 months to complete its life cycle in a 'seed-to-seed' production system. *Alternaria radicina* is a seed- and soil-borne fungal pathogen of carrot seed crops (CABI/EPPO, 1998) which is most prevalent on older plants and senescing leaf tissues which touch the soil surface (Meier *et al*., 1922; Soteros, 1979; Pryor, 2002). In winter, young carrot plants become susceptible to *A. radicina* infection, as senescing leaves touch

surrounding contaminated soil. The pathogen produces conidia on necrotic and senescent leaf tissue, which are then spread by wind, overhead irrigation, and splashing rain water (Neergaard, 1977; Pryor, 2002), causing petiole infection and leaf blight as well as seed infection (Grogan and Snyder, 1951; 1952; Tylkowsa, 1992).

In summer the black necrotic lesions produced on petioles provide avenues for the infection of root crowns and tap roots which then develop black rot symptoms on the shoulder regions (Grogan and Snyder, 1952; Farrar *et al*., 2004). Wind-borne conidia then move through the crop and infect umbels. After harvest of the carrot seed crop, *A. radicina* can survive in dead plant debris and its conidia can remain viable for more than 8 years in the soil in the absence of a host plant (Maude and Shuring, 1972; Maude and Bambridge, 1991 (cited in Farrar *et al*., 2004).

Heat has long been used as a method to control weeds (Atkinson, 1995) and pests and diseases (Hardison, 1976; Skoglund *et al*., 1999).

Thermosanitation can control some soil-borne diseases by killing the resting structures of the pathogens, although the effectiveness depends on the heat that can be applied and depth to which it can penetrate the soil (Newhall, 1955; Bollen, 1985). The use of heat to control pathogens in seeds and harvested crops (e.g. carrot roots) has been effectively applied for many years (Farrar *et al*., 2004), but its ability to control foliar pathogens has been little investigated.

This paper reports the effect of flaming and steaming treatments, applied in winter, with the aim of destroying foliar borne inoculum of *A. radicina*, thereby potentially reducing black rot development in commercial carrot seed crops.

Materials and Methods

First Year Experiment (2006)

Four commercial hybrid carrot seed fields in mid-Canterbury were chosen for thermal trials. Flaming treatments were applied at all sites, while steaming treatments were applied at two sites. As flaming was originally the major method being investigated, the flaming treatments were replicated four times, and for observation, the steaming treatments twice. Each experimental plot was 15 x 3 m. The tractor driven flame burner and steamer were described by Merfield (2006) and Merfield *et al*. (2009). Treatments, which were applied on 6 September 2006, were tractor speeds of 1.7, 2.3 and 2.9 km h^{-1}. The plants not heat treated in each farm were used as controls.

The effects of flaming and steaming on carrot plant regrowth were assessed by visual observation of treated plants and untreated plants at regular intervals. Carrot plant dry matter accumulation was determined in December 2006. Whole plant samples were collected from a randomly selected 1 m row section. The plants were washed under tap water to remove soil and other debris, cut at the base of the shoot with a sharp knife, and the roots and shoots weighed separately. Both roots and shoots were dried at 70 °C to constant dry weight; this was determined by removing the samples from the oven every day and weighing. From the final dry weights, total dry matter weight (kg ha^{-1}) was calculated.

Assessment of black rot disease on roots

In January 2007, 10 carrot plants were uprooted from each plot and washed under tap water. Air dried tap roots were visually assessed for black rot infection, using a 0 to 4 rating scale where: 0 = healthy; 1 = 1-25% of the shoulder region blackened due to black rot; 2 = 26-50% of the shoulder region blackened due to black rot; 3 = 51-75% of the shoulder region blackened due to black rot; 4 = > 75% of the shoulder region blackened due to black rot.

Assessment of foliage disease

Ten carrot plants per plot were tagged and inspected for foliage infection every month after application of thermal treatments (until the difference between treated and untreated plants became non-significant) using a 1 to 10 rating scale where: 1 = no infection; 2 = infection on lower leaves; 3 = infection on lower stem and lower leaves; 4 = infection on lower stem and lower leaves senesced; 5 = infection on middle stem and leaves; 6 = infection on middle stem and all leaves; 7 = infection on upper stems and all leaves; 8 = leaves senesced and infection on upper stems; 9 = leaves senesced and most of the stem diseased and 10 = leaves senesced and all of the stem diseased (Merfield, 2006).

Second Year Experiment (2007)

The steaming trial was repeated in 2007 at one mid-Canterbury site, using the same tractor speeds as in 2006, but two different dates (5 June and 20 July, 2007). Disease and dry matter weight assessment were made as before.

Seed infection was assessed at maturity, in April 2008, using 10 primary umbels hand harvested from each plot. Seeds were dried at 30 °C to 8% seed moisture. Seed was then hand removed from dried umbels and cleaned by hand-rubbing to remove spines. Seeds were then thoroughly mixed. Hands were washed in 70 % ethanol between the cleaning of each sample to reduce the chance of contamination. Seed infection was assessed by plating 100 carrot seed $plot^{-1}$ onto a semi-selective agar (ARSA) (Pryor *et al*., 1994), and incubating in the dark at 27 °C for 14 days. After incubation, seeds which had produced distinguishable black hyphae of *A. radicina* were counted as infected seeds and those that did not as non-infected (Pryor *et al*., 1994).

Statistical Analysis

Data were checked for normal distribution, by the Shapiro-Wilk Test using Genstat Edition 12, and were square root transformed where required. Statistical analysis was performed on raw data when normally distributed and on transformed data when not normally distributed. For the latter, back transformed results are presented.

In 2006, as each experimental site had a different growing environment and carrot hybrids black rot infection data at each site or foliage infection at each assessment month at each site were analysed separately.

In 2007, foliage infection data at each assessment date, for each treatment application, were separately analysed using one-way ANOVA because the assessment dates for each treatment time differed. However, black rot infection data were analysed using two-way ANOVA to determine individual as well as interactions between treatments. The effect of the two treatment timings on

black rot infection was analysed through a paired t-test. Treatment means were separated by using Fisher's LSD at a 5% significance level. All statistical computations used Genstat Edition 9.

Results

First Year Experiment (2006)

The flaming treatment burnt carrot foliage immediately while steaming darkened it initially, but after 24 hours the foliage developed a similar burnt appearance. Carrot plants regrew foliage such that, by 60 d after the thermal treatment, growth was similar for both flame and steam treated and control plants. By December there was no significant difference (P>0.05) in total carrot dry matter between treated and untreated plots (data not presented) which suggested that flaming and steaming had no adverse effects on plant productivity. Although not measured, there were no visual differences in time of flowering or umbel density.

Infection of new foliage was initially reduced by the treatments (P≤0.05), but by three months mature leaves had a similar disease incidence to the untreated controls (Figure 1, 2). At all three speeds, steaming reduced (P≤0.05) black rot disease ratings on carrot roots but flaming did not (Figure 3).

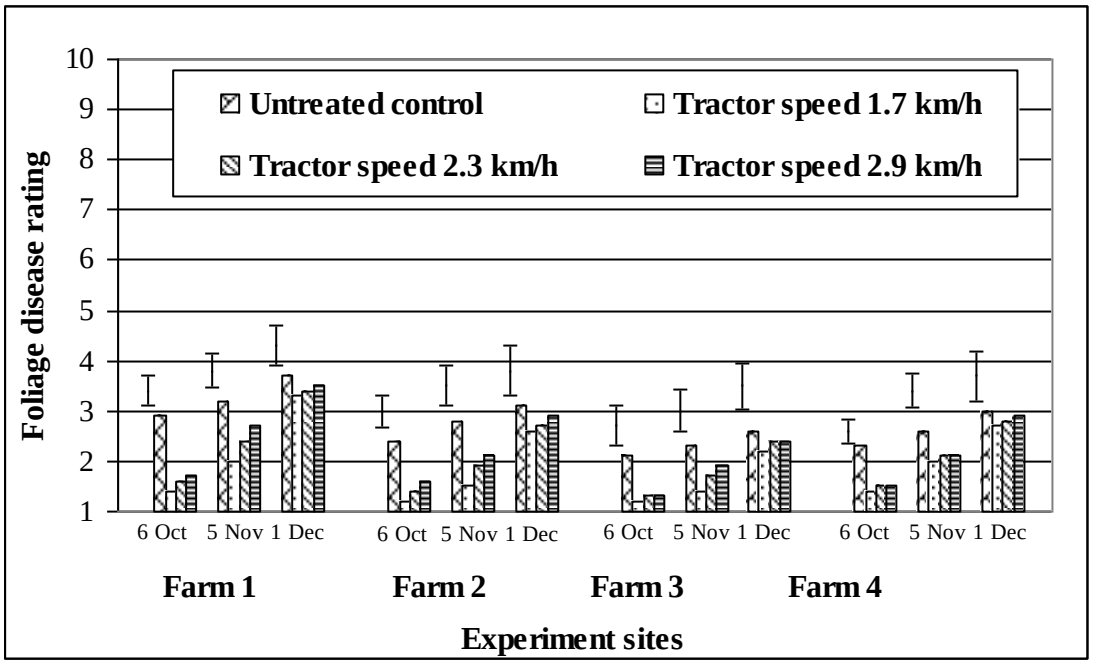

Figure 1: Effect of flaming on carrot foliage infection caused by *A. radicina* in 2006. Statistical analysis at each farm at each assessment date was done separately. Bars indicate LSD values (P≤0.05).

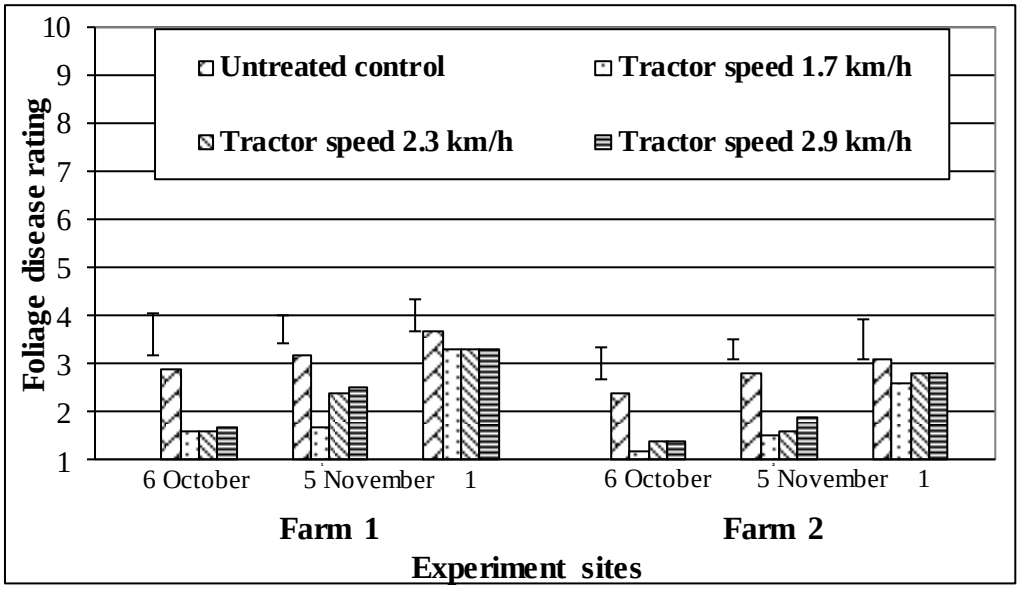

Figure 2: Effect of steaming on carrot foliage infection caused by *A. radicina* in 2006. Statistical analysis at each farm at each assessment date was done separately. Bars indicate LSD values (P≤0.05).

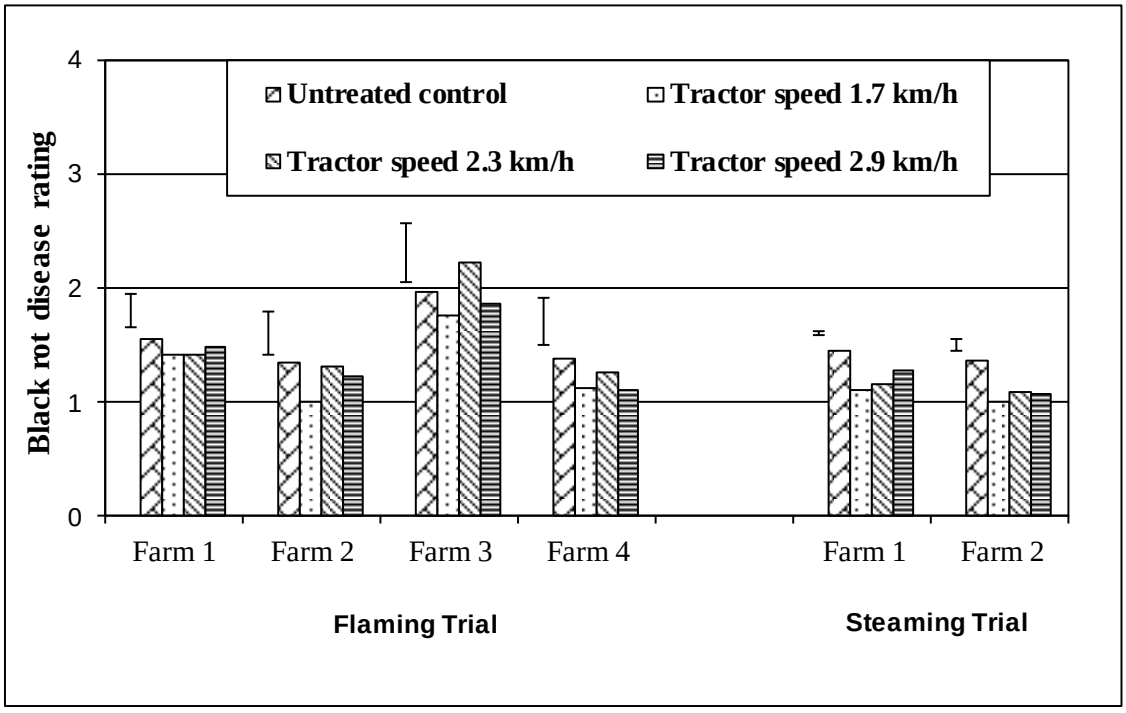

Figure 3: Effect of flaming or steaming on subsequent black rot disease expression in carrot roots in January 2007. Statistical analysis at each farm was done separately. Bars indicate LSD values (P≤0.05)**.**

Second Year Experiment (2007)

Treated carrot plants took 80 days to recover their growth, and again there was no adverse effect on dry matter production when assessed in December (data not presented), which confirmed that steaming has no adverse effect on normal carrot crop growth.

Foliage disease ratings were again initially reduced by steaming ($P \leq 0.05$; Figure 4). Carrot plants treated in the first week of June had a significantly lower disease rating for three months compared with only two months for the third week of July application (Figure 4).

Steaming at 2.3 km h^{-1} and 2.9 km h^{-1} in June 2007 gave significantly ($P \leq 0.05$) better control of black rot disease compared with steaming at 1.7 km h^{-1} and the control (Table 1). Steaming in June gave a significantly ($P \leq 0.05$) better disease control than steaming in July. Steaming had no significant effect ($P \leq 0.05$) on the percentage of seeds (mean = 14%) infected by *A. radicina* (data not presented).

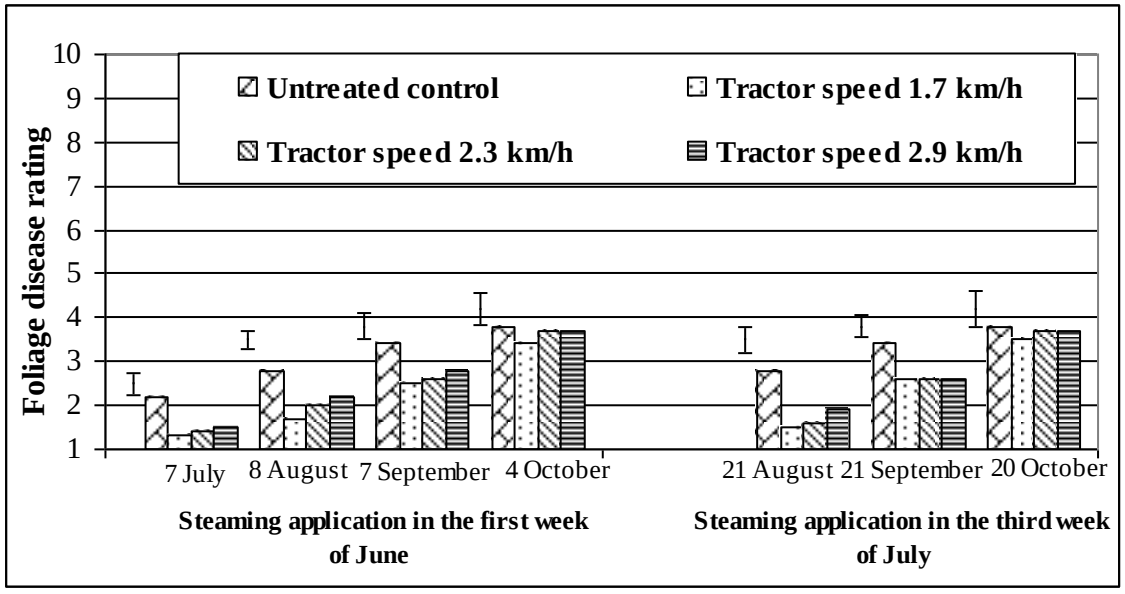

Figure 4: Effect of date of steaming on carrot foliage infection caused by *A. radicina* in 2007. Statistical analysis at each assessment date at either treatment application date was done separately. Bars indicate LSD values ($P \leq 0.05$).

Table 1: Effect of steaming on black rot disease in carrot roots assessed in January 2008.

Speed of steamer	Time of steaming*		Mean black rot disease rating at different steamer speeds**
	First week of June 2007	Third week of July 2007	
Control	1.33 c	1.33 c	1.33 h
1.7 km h^{-1}	1.32 c	1.32 c	1.32 h
2.3 km h^{-1}	1.07 a	1.30 c	1.18 k
2.9 km h^{-1}	1.16 ab	1.26 bc	1.21 k
Mean disease rating at different times***	1.21 s	1.30 t	

*For comparison of interaction of steamer speed by time of application.
**For comparison of mean value of disease rate at different speeds of steamer.
***For comparison of mean values of disease rating at different times.
Mean values followed by a different letter are significantly different according to the LSD test ($P \leq 0.05$).

Discussion

Merfield (2006) applied thermal treatments (flaming and steaming at 2 km h^{-1}) to pot grown (from seed) two month old carrot plants artificially inoculated with *A. radicina*, *A. dauci* and *Cercospora carotae* and reported complete elimination of all of the pathogens. In the field, in both seasons, flaming and steaming both significantly reduced, but did not eliminate *A. radicina* for two to three months after treatment. However, subsequent infection levels did not differ from that of the untreated plants. This was not unexpected, as thermally treated plots were surrounded by the remainder of the commercial crops, from which inoculum would have spread.

Merfield (2006) demonstrated that once carrots had reached the 6 leaf stage (approximately 8 cm in height) they survived thermal treatment, because as rosette-forming plants, both the apical and axillary meristems are protected by petioles that are often thickened. Merfield (2006) also found that thermal treatment of carrots after the transition from vegetative to reproductive growth negatively impacted on carrots by checking plant growth, reducing height, delaying the onset of flowering, and reducing seed yield. Thermal treatment of still vegetative carrot plants in mid/late winter had no permanent effect on subsequent plant growth. Two or three months after treatment there were no obvious visual differences between treated plots and the control. By December, total plant dry matter from treated plots did not differ from the control.

Flaming had no effect on black rot, but steaming, in both years, significantly reduced occurrence of the disease. Steaming is considered more lethal than flaming (Ascard *et al*., 2007) due to the considerably larger latent heat of condensation/vaporisation of water compared with the specific heat of dry air, and less heat loss via evaporation and/or transpiration compared with open

flames (Sirvydas *et al*., 2002; Merfield, 2006).

Black rot control was not affected by tractor speed for the steaming treatment in the first year. However, in the second year control was better at the two faster application speeds. The reason for this is unknown but it is possible that at 1.7 km h^{-1}, steaming injured the carrot plants and made them more susceptible to *Alternaria radicina* infection. Timing of steaming was important, as a July application gave no disease control. However, even for a June application, while the reductions in black rot were significant, they were not large, and no treatment completely controlled the pathogen.

The failure of steaming to provide long-term control of seed borne *A. radicina* infection was not unexpected. The experimental plots were in a small area of a commercial field where surrounding plants would have provided wind-borne inoculum of the pathogen which then spread to the trial plots.

Carrot plants can be infected by *A. radicina* from at least three sources; soil, seed and wind-borne conidia. By winter or early spring, crops are already infected, and pathogen control at this time should result in a reduction in disease levels. Steaming has the potential to provide this control. However whether the potential can be turned into practise will require further investigation using much larger plots and an experimental design which seeks to minimise the opportunity for inoculum to move from control to treated plots.

In carrot seed production, the aim during vegetative growth is to reduce *A. radicina* inoculum to as low a level as possible, which subsequently will help to reduce umbel infection. Severely infected umbels may produce no seed, but more commonly produce infected seeds. This can result in the failure of a seed lot to meet contracted germination standards. In both cases the grower faces economic losses. For organic carrot seed production, if steaming is to be used as a method of weed control (Merfield, 2006), then a reduction in *A. radicina* (and other pathogens such as *A. daucii* and *Cercospora. carotae*) could be an additional benefit.

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