Organic F1 Hybrid Carrot Seed (*Daucus carota* L.) Production: The Effect of Crop Density on Seed Yield and Quality, Thermal Weeding and Fungal Pathogen Management.

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Dedicated to the memory of

Derek Peter Merfield

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Organic F1 hybrid carrot seed (*Daucus carota* L.) production: the effect of crop density on seed yield and quality, thermal weed and fungal pathogen management.

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There is interest among vegetable seed companies in the production of certified organic seed, primarily because of USA and EU legislation. Companies and farmers growing organic carrot seed in Canterbury, New Zealand have encountered a number of production issues, including appropriate crop densities, fungal pathogens, particularly *Alternaria radicina*, and weed management of overwintered intrarow weeds.

To determine suitable densities for organic carrot seed crops, the effect of crop densities of 5, 11, 20, 55 and 82 plants m^{-2} on two F1 parent seed parent cultivars with contrasting morphologies was studied. There were large differences between the cultivars with respect to height, yield, yield response to density, germination, germination response to density and thousand seed weight (TSW). Yield varied between cultivars and among umbels with yield increasing with increasing density for cv. B but not A. TSW varied between cultivars and umbel levels. Seed vigour varied between umbel levels and *A. radicina* seed infestation increased with increasing density. The hypothesis stated in the literature that higher crop densities increases seed quality in commercial crops is supported by these results. It is also suggested that in order to correctly test the hypothesis, the practice of separating the umbel levels is methodologically incorrect.

A direct-fired steam weeder was developed, to avoid the practice used in standard boiler designs of superheating pressurised water to produce steam. This approach makes it possible to safely generate sufficient amounts of steam using tractor mounted equipment to effectively kill weeds. Steam weeders have a range of advantages over flame weeders including improved heat transmission due to the latent heat of condensation of

steam and by stopping evapotranspirational cooling of plants after treatment. The direct-fired steam weeder also poses no fire risk, is unaffected by wind and is powered by raw vegetable oils, biofuels or diesel. This is offset by greater complexity, weight, use of large volumes of water and higher cost compared with flame weeders.

Tests on a range of weeds at various growth stages revealed that springtime thermal weeding of overwintered intrarow weeds is unlikely to kill most weed species. The dose response curves commonly used for optimising herbicide usage are less suitable for determining the optimal thermal doses to kill weeds. Instead, the transfer of heat onto and though a plant's tissues to the hypocotyl stem and/or aerial meristems should be modelled.

Although springtime thermal treatment of intrarow weeds proved of limited use for weed control, it may have potential to eliminate foliar fungal pathogens. In a glasshouse experiment, the foliage of pot-grown carrots was artificially infested with *A. radicina*, *Alternaria dauci*, and *Cercospora carotae*. Thermal treatment caused complete disinfestation for all fungal pathogens. This effect needs to be confirmed under field conditions, and the impact of defoliation on carrot plant growth, seed yield and seed quality has to be established.

Five biological control agents (BCA), *Bacillus subtilis*, *Trichoderma viride, Trichoderma atroviride, Rhizobium rhizogenes* and Effective Microorganisms (EM) were tested for their ability to control *A. dauci*, *A. radicina* and *C. carotae* in a sequence of trials including *in vitro* laboratory assessments as well as *in vivo* glasshouse and field trials. Initial laboratory work showed that some BCA inhibited pathogen growth. However, glasshouse and field trials found no evidence that the biocontrol agents could satisfactorily control the three pathogens or other carrot infesting fungi.

Overall, the study confirmed current crop densities and spacings as suitable for organic carrot seed production, and that higher plant densities may increase pathogen infestation of seed. The BCA failed to control the fungal pathogens in the field; however, thermal weeders show promise for managing foliar fungal pathogens.

Keywords: organic agriculture, F1 hybrid, seed production, seed quality, *Daucus carota*, crop density, weed management, thermal weeding, steam weeding, flame weeding, *Alternaria dauci*, *Alternaria radicina, Cercospora carotae*, *Bacillus subtilis*, *Trichoderma viride, Trichoderma atroviride, Rhizobium rhizogenes*, Effective Microorganisms.

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Contents

List of Tables

List of Figures

Chapter 1. Introduction

Organic agriculture is now a well established and clearly defined approach to primary production and associated industries (Lampkin *et al.*, 2004). Organic production is experiencing growth of about 20% per year and has a current value of approximately NZ$40,000,000,000. Some 26,000,000 ha and 559,000 farms are managed organically worldwide and this represents c. 2.3% of world agriculture. It is officially recognised by many governments and intergovernmental organisations and is increasingly viewed as a valuable means to address a range of problems associated with 'industrial' agriculture (Pretty *et al.*, 2000; Fookes, 2001; Heaton, 2001; Campbell & Ritchie, 2002; Lampkin *et al.*, 2004; Willer & Yussefi, 2005). However, the exclusion of practically all synthetic pesticides, herbicides and fungicides by organic production regulations creates a number of novel problems for crop production that need addressing.

There is considerable interest among vegetable seed companies, both worldwide and in New Zealand, in producing certified organic seed. This is primarily because, on 31 December 2003, the derogation to EC Regulation 2092/1991 allowing the use of non-organic seed in organic production systems was to end (Anon, 1991; Lampkin, 1994). However, it became clear that there would be insufficient organic seed available for a considerable range of crops by the end of 2003, so EU Regulation (EC) No. 1452/2003 extended the derogation and required the creation of country-specific databases to regulate the use of seeds and seed potatoes in organic farming (Anon., 2003). This system of databases and the supporting legislation is designed to facilitate the transition to full use of organic seed in organic production systems.

The Canterbury province, situated on the east coast of the South Island of New Zealand is a globally significant area for seed production. Local seed companies began investigating growing carrot (*Daucus carota* L. var. *sativus* D.C.) seed organically in 1999. They were among the first in the world to attempt this and a number of production problems were encountered. Three issues were identified by the author, Midlands Seed Ltd. (a local seed company interested in organic seed production), Prof. J. Hampton (Lincoln University) and Mr T Chamberlain (the organic grower contracted to grow organic seed crops for Midlands Seed Ltd.) that needed to be addressed before organic carrot seed production in the Canterbury region could progress; viz:

- Suitable densities for organic crops;
- Weed management, particularly intrarow weeds in overwintered steckling crops;
- Management of fungal pathogens, particularly *Alternaria radicina* Meier, Drechler & Eddy.

These three key issues form the basis for the research in this thesis and are addressed in individual chapters (detailed below). In addition, Chapter 2 is a literature review and Chapter 6 concludes the thesis with a general discussion that unites all the research topics and relates them to the progress achieved in commercial organic carrot seed production.

1.1. Crop Density

While the effect of plant density on carrot seed production has been a focus of research for over fifty years (e.g., Franklin, 1946), the results of altering plant densities have often been variable and sometimes contradictory. The main effects that are sought from higher plant densities are increased seed yield and quality and a reduction in the variation of root size in the following carrot root crops (Willey & Heath, 1969; Salter *et al.*, 1981). Accepting the above ecological restraints, the rationale for increased plant densities improving the above three attributes is as follows:

- The relationship between increased seed yield and increasing plant populations has been clearly demonstrated for a wide range of crops, with yields either increasing initially followed by reaching a plateau at higher densities, or increasing and then decreasing at higher densities (Willey & Heath, 1969);
- Carrot seed quality is expected to rise because increasing plant densities increase the proportion of primary umbels; seed from primary umbels is larger, more vigorous and produces larger seedlings than does seed from lower order umbels (Jacobsohn & Globerson, 1980);
- Variation in the size of carrots in a root crop is mostly a result of initial size differences between seedlings and the time of emergence, which are in turn mostly related to seed variability (Currah, 1978).

The current plant densities of approximately 20 plants m^{-2} used in Canterbury carrot seed crops have been optimised for non-organic production systems and may not be suitable for organic production. All but one of the published trials studying the effect of plant density on carrots have tested only one cultivar. However, F1 parent lines vary considerably in their morphology and other growth aspects, so the response to varying

crop density may differ among cultivars. Different planting densities may also alter crop microclimate and thus conditions for growth and spread of fungal pathogens.

The experiments presented in Chapter 3 address the issue of crop density. The effects of altering crop density for two F1 seed parent cultivars, which differed in the number of lower order umbels were compared. Seed yield and quality for each umbel level were determined. Seed quality measurements comprised thousand seed weight (TSW), germination, vigour and infestation by the common carrot pathogens *A. radicina* and *Alternaria dauci* Kuhn, Groves, and Skolko.

The aims of the research presented in this chapter were:

- To establish the effect that varying plant density has on carrot seed yield and quality;
- To determine whether two cultivars with contrasting growth habits differ in their response to varying plant densities in terms of seed yield and quality;
- To determine the contribution of the different umbel orders to seed yield and quality for both cultivars at different densities.

1.2. Weed management

The biennial nature of carrot seed production also presents novel weed management issues. In seed-to-seed carrot production in New Zealand, the crop is sown in February and grown over the winter. The Canterbury climate is sufficiently cold and wet during winter to prevent access to fields and makes mechanical control of weeds ineffective. In-crop weed management in organic production systems is primarily achieved by physical methods, which in row crops such as carrots is usually done by interrow hoeing. When used correctly, these are very effective and can completely eliminate weeds in the interrow area, even those that have established and grown during winter. However, over-wintered weeds in the intrarow area are considerably more problematic. Although there are now a number of intrarow weeder designs, e.g., torsion weeders, that are effective against weed seedlings, they have limited effects on larger overwintered weeds (Welsh *et al.*, 2002). This leaves hand weeding as the main control method. However, this is often expensive and finding suitable labour can be difficult. Alternative approaches to controlling large intrarow weeds are therefore required. Thermal weeding, e.g., flame weeding, is one option as it has the potential to kill intrarow weeds but not the carrot crop, if there is sufficient difference between the carrots' and the weeds' susceptibility to heat.

Although flame weeding is a valuable tool for growers, it also suffers from a range of problems including very low efficiencies, fire hazard, large reductions in effectiveness in windy conditions and the use of liquefied petroleum gasses (LPG) as fuel, which is inconvenient for farmers (de Rooy, 1992; Ascard, 1994; Palmer, 1996). Steam has been shown in laboratory tests to be a superior means of transmitting heat into plants when compared with air. This is mainly due to the latent heat of condensation, but it is also a result of steam preventing the evapotranspirative cooling of plants that occurs with flame weeding (Sirvydas *et al.*, 2002; Andreas Bertram, pers. comm. 2005). However, generating steam on tractor-mounted equipment has to date been problematic. Most steam boilers are designed for static use and are unsuitable for carriage by tractors. Two steam weeders that use a modified pressurised boiler design are in commercial production and use. However, these also have problems, including the need for safety systems to prevent a superheated steam explosion, fixed heat outputs and combining steam generation and application, which limit their ability to be effectively used in different production systems, e.g., vegetables vs. pip fruit. An alternative steam generation approach was developed, here described as 'direct-fired steam', which avoids the need for a pressure vessel by spraying water as a fine mist directly into the exhaust gasses from a burner. This method has the further advantages that a wide range of heat outputs can be produced and, as steam generation and application are separated, it can be tailored to a wide range of uses. In addition, steam weeders have many advantages over flame weeders; for example, they are more efficient, pose a low fire risk, are unaffected by wind and can burn diesel or biofuels rather than LPG.

The experiments described in Chapter 4 address the issues of thermal weeding by comparing the advantages and disadvantages of flame and steam weeders and detailing the design of the direct-fired steam weeder. In addition, the ability of a flame and the direct-fired steam weeder to kill small weeds were compared over a range of travelling speeds, and their effect on carrots and twelve weed species at a range of growth sizes was tested. Finally, the effect of flame weeding carrots plants at the point of bolting on seed yield and quality was studied.

The aims of the research presented in this chapter were:

- To briefly review alternative thermal weeding approaches;
- To describe the direct-fired steam weeder;
- To compare advantages and disadvantages of flame and steam weeders;

- To determine if the direct-fired steam weeder is more effective than a comparable flame weeder by testing their efficiency in killing a surrogate weed between the cotyledon stage and the two true leaves stage over speeds of 3 to 7 kph;
- To compare the effect of steam and flame weeders on carrots and a range of weed species at five growth stages;
- To discuss the appropriateness of using dose response curves as a basis of predicting a thermal weeder's capacity to kill larger weeds, and the role morphology plays in determining a plant's susceptibility to thermal weeding;
- To ascertain the impact of flame weeding carrot plants at bolting on carrot seed yield and quality.

1.3. Fungal pathogen management

A. radicina is an increasing problem in both organic and non-organic carrot seed crops in Canterbury (James Smith, pers. comm. 2005) as existing synthetic fungicides appear to be losing their effectiveness. Moreover, organic producers are unable to use such products and have access to only a very limited number of non-synthetic fungicides, principally sulphur and copper compounds (Anon., 2001). This restricts them to growing F1 parent lines that have good disease resistance, which considerably limits the range of F1 crosses they can produce. Two other fungal pathogens *A. dauci* and *Cercospora carotae* (Pess.) Solheim are also present in Canterbury, although they appear to be considerably less prevalent and problematic than *A. radicina*. An integrated management approach is, therefore, required to address the increasing levels of *A. radicina* and to control *A. dauci* and *C. carotae*, where they occur.

An increasing number of microbial biological control agents (BCA) are being registered for fungal disease control, both in New Zealand and worldwide. Some of these are pathogen specific while others have a broad spectrum of activity. Previous research, mostly laboratory based, indicated that BCAs approved for agricultural use in New Zealand may have the potential to control *A. dauci*, *A. radicina* and/or *C. carotae* (Besson & Michel, 1987; Catska, 1989; Sesan, 1990; Hentschel, 1991; Castro *et al.*, 1993b; Higa, 1994).

All three pathogens are known to infest seed and only *C. carotae* has not been found within seeds (Thomas, 1943; Scott & Wenham, 1973). Synthetic fungicidal seed dressings that have been used effectively in non-organic production for controlling seed borne fungi are banned in organic production and alternatives are required. Soaking seed in hot water has been shown to reduce *A. dauci* and *A. radicina* infestation levels

in carrot seed (Strandberg & White, 1989). Most research studying hot water treatment effects on seed quality use standard germination tests (ISTA, 2006) to determine which temperature and treatment duration are safe to use. However, hot water treatment has a number of similarities with the accelerated ageing seed vigour test (ISTA, 2006), principally exposure to elevated moisture and temperatures. It is possible that damage to seed is occurring that is not being detected by the standard germination tests.

When thermal weeders are used after crop emergence (as described in section 1.2) the carrot's foliage is completely killed. It is, therefore, likely that any fungal pathogens present on the leaves would also be killed. If the pathogens are restricted to the carrot foliage during the winter (as opposed to the root or soil) then thermal treatment during winter or spring may offer a physical means to reduce pathogen levels in crops. This could delay the re-establishment of the pathogens and thus lead to a reduction of the number of fungicide applications required for both organic and non-organic crops. If the technique could eliminate or reduce pathogens to very low levels in crops, then re-infestation from external sources could become of increased importance. Crop debris from previous crops in nearby fields could be a significant source of conidia to infest 'clean' crops, so the prevalence and persistence of the pathogens in commercial fields needs to be established. The experiments outlined in Chapter 5 address the management of *A. radicina, A. dauci* and *C. carotae*. Firstly, the effect of hot water treatment on carrot seed quality using germination curves was established followed by a study of optimum temperature and treatment duration for reducing *A. radicina* infestation.

The ability of existing BCA to control the pathogens was established in a sequence of experiments. Growing media suitable for culturing the pathogens as well as BCA and maximising pathogen sporulation were identified. The ability of the BCA to control the pathogens was established *in vitro*, in pot trials under controlled conditions and in a field trial. The BCA agents were also tested for compatibility *in vitro* to see if they could be used in a mixture rather than separately, which is the standard approach used at present.

In addition, a preliminary study of the presence of *A. radicina* and *A. dauci* on crop debris from commercial fields was undertaken and the ability of thermal weeders to disinfest artificially infested carrot plants in controlled conditions was investigated.

The aims of the research presented in this chapter were:

- To establish maximum hot water treatment times and durations for carrot seed and optimum treatment duration and temperature to reduce *A. radicina* in carrot seed;
- To determine whether more information on seed quality than can be provided by the standard germination test is required to determine when hot water seed treatment may start to impact negatively on carrot seed;
- To find media suitable to test the BCA's ability to control *A. dauci, A. radicina* and *C. carotae in vitro*, under controlled conditions and in the field;
- To determine the best media and inoculation technique for pathogen spore production;
- To undertake a preliminary survey of the abundance of *A. radicina* and *A. dauci* on carrot seed crop debris in commercial fields;
- To establish if thermal treatment could reduce occurrence of *A. dauci*, *A. radicina* and *C. carotae* on carrots under controlled conditions.

Chapter 2. Literature review

2.1. The effect of crop density. Some agronomic influences on carrot seed yield and quality

2.1.1. Introduction

While the effect of planting density on carrot seed production has been a focus of research for over fifty years (e.g., Franklin, 1946), the results of altering plant densities have often been varied and sometimes contradictory. The main effects that are sought from higher plant densities are increased seed yield and quality, and a reduction in the variation of root size in the following carrot root crops (Willey & Heath, 1969; Salter *et al.*, 1981). The rationale for increased plant densities improving the above three attributes is as follows:

- The relationship between increased seed yield and increasing plant populations has been clearly demonstrated for a wide range of crops, with yield either initially increasing then reaching a plateau at higher densities, or increasing then decreasing at higher densities (Willey & Heath, 1969).
- Carrot seed quality is expected to rise because increasing plant densities increase the proportion of primary umbels; seed from primary umbels is larger, more vigorous and produces larger seedlings than seed from lower order umbels (Jacobsohn & Globerson, 1980).
- Variation in the size of carrots in a root crop is mostly due to initial size differences among seedlings and the time of emergence, which are in turn mostly due to seed variability (Currah, 1978).

In commercial crops, harvesting occurs when the seed on the primary umbel has matured but before it starts to shatter. In carrots each umbel order flowers at different times, with the primary umbel flowering about two weeks before the secondaries, the tertiary flowering a while later and so on. This has the follow-on effect that the seeds on different umbel orders mature at different times (Gray & Steckel, 1985). Seeds at different stages of maturity vary considerably in their rate of germination, percentage germination and seedling size. When seeds from different umbel orders at different stages of maturity are mixed together at harvest, this will produce a wide variation of seed quality within seed lots. Increasing plant populations can suppress the production of lower order umbels, and reduce the amount of seed they produce, considerably

reducing their contribution to the harvest. This will reduce the amount of variation within seed lots, which should result in more even root crops (Gray & Steckel, 1980).

However, altering plant density has a number of effects on other areas of production. One effect of altering density is to modify the crop microclimate which can in turn affect disease levels (Tompkins *et al.*, 1993; Fernandez *et al.*, 2002). For example, the onset of the aerial stem rot *Erwinia carotovora* (Jones) Bergey *et al.* subsp. *carotovora*, in potatoes (*Solanum tuberosum* L.) was between 5 to 34 days earlier for densities of 5,356 plants ha^{-1} than 1,339 plants ha^{-1} (Cappaert & Powelson, 1990). *A. dauci, A. radicina* and *C. carotae* all require very high relative humidity (RH) or surface water for several hours, normally overnight, to both infect carrots and sporulate (Lauritzen, 1926; Strandberg, 1977; Carisse & Kushalappa, 1992). Higher planting densities are likely to result in reduced airflow within the crop leading to increased RH and longer periods of leaf wetness. This in turn could result in higher disease levels and/or earlier disease onset. Alternatively, conidia require wind speeds of 2 to 3 m s^{-1} to be released from the conidiophore (Strandberg, 1977). As crop densities increase, wind speed within the crop canopy may be sufficiently reduced to repress conidia release and have a disease suppressing effect.

Therefore, there is a potential conflict between increasing plant density to increase yield and quality and decreasing plant density to improve disease control.

2.1.2. Inconsistencies in the results of plant density trials

As discussed above, the use of higher plant densities to achieve the objectives of increasing seed yield and quality and decreasing root crop variation has produced inconsistent and even contradictory effects, even to the extent of conflicting results by the same researcher. For example, there was no change in seed weight for cv. Supreme Chantenay Red-Cored between densities of 10 to 80 plants m^{-2} for steckling to seed production, and 11 to 256 plants m^{-2} for seed to seed production (Gray, 1981), but seed weight was greater in crops grown at lower densities (10 plants m^{-2}) than higher densities (80 plants m^{-2}) over two years for cv. Chantenay Red-Cored selection Royal Chantenay (Gray *et al.*, 1983). Conversely, later studies using the same cultivar reported that mean weights per seed were greater in the high density (80 plants m^{-2}) than low density (10 plants m^{-2}) crops (Gray & Steckel, 1983a). This kind of variation and contradiction is found in a range of other measurements including crop height, flowering date, spread of flowering, differences in the umbel orders and even yield. For example, cv. Chantenay Red-Cored selection Royal Chantenay displayed an increased

flowering spread for the primary umbel with increasing densities of stecklings between 10 to 80 plants m^{-2} (Gray & Steckel, 1985). In contrast the time or duration of flowering of cv. Danvers was unchanged over densities of 2 to 25 plants m^{-2} (seed to seed) (Oliva *et al.*, 1988) while there were no significant differences between flowering times of primary umbels at any density or between seed to seed or steckling production for densities of 10 to 256 plants m^{-2} (Gray, 1981). The cv. Puda Kesar showed no yield difference between 2.7 to 11 plants m^{-2} (Sharma & Singh, 1981), while the cv. Local Sel yielded the most at the highest density (2.78 vs. 11.1 plants m^{-2}) (Lal & Pandey, 1986). In comparison the maximum yield occurred at 12 plants m^{-2} over a density range of 2 to 36 plants m^{-2} (Oliva *et al.*, 1988), while yield increased by 50 to 55% from 10 to 80 plants m^{-2} for two years but not a third, with yields ranging between 966 to 2376 t ha^{-1} (Gray *et al.*, 1983).

The situation is also confused concerning seed quality. For example germination, germination rate, seedling growth, embryo length and abnormal seedlings within each umbel order were unaffected by plant densities between 2 to 25 plants m^{-2}, but when averaged over all umbels the high density crops produced seedlings with longer radicles, lower coefficient of variation (CV) of radicle length and fewer abnormal seedlings. However, the trend was reversed for densities of 36 plants m^{-2} where quality decreased and the number of abnormal seedlings increased from17 to 29% (Oliva *et al.*, 1988). In comparison there was an increase in the spread of emergence and CV of seedling weight for seeds from the primary umbel of cv. Chantenay Red-Cored selection Royal Chantenay for increasing densities of 10 to 80 plants m^{-2} (Gray & Steckel, 1980).

It therefore appears that there are a number of other factors that moderate the effect of density on yield and seed quality. These may include cultivar, climate, production method (seed to seed vs. stecklings), and harvest time.

2.1.3. Causes of inconsistent density trial results

All the above studies except one used a single cultivar and the studies often used different cultivars. There is considerable variation in plant morphology and growth responses among carrot cultivars. For example, some cultivars produce few lower order umbels regardless of plant density while others continue to produce them at higher densities (James Smith, pers. comm., 2003). Therefore, cultivars may differ in their response to increasing density. The results from the one trial which did compare two cultivars (at the same but unspecified densities) indicate that this can be the case, as there was a clear difference in rate of germination of seed from primary vs. secondary

umbels in cv. Jawa but not in cv. Koral (Szafirowska, 1994). While different cultivars can vary in their response to changing density, single cultivars can also produce divergent results; for example, yield increased with increasing density in identical, field grown crops in two years but not in the third (Gray & Steckel, 1983a). The cause of this result was undetermined but comparisons with the same cultivar produced in polytunnels indicated climatic factors were responsible. The carrot density trials reviewed for this research were conducted in a range of countries, including England, Israel, the USA and New Zealand, the climates of which range from temperate through Mediterranean to arid (Pearce & Smith, 1990). If density effects are moderated by climate, then this might be a reason for some of the variation among trials.

Despite the variation in results from density trials, some agreement within the literature can be ascertained. Increasing plant densities increase the proportion of primary umbels and thus the proportion of seed that comes from primary umbels (Gray & Steckel, 1980; Jacobsohn & Globerson, 1980; Oliva *et al.*, 1988; Satyaveer *et al.*, 1994). Increased densities also decrease the number of umbels and quantity of seed produced per plant (Gray, 1981; Gray *et al.*, 1986; Oliva *et al.*, 1988; Evans, 2000; Ramnut & Thakan, 2002). Generally yield increases with increasing densities (Gray, 1981; Gray *et al.*, 1983; Lal & Pandey, 1986; Oliva *et al.*, 1988).

2.1.4. The effect of harvest date on seed quality

Harvest date has been shown to have a significant effect on seed quality, and compared to the measurements taken as part of the density trials (discussed above) there is much better agreement between the results of different experiments. Percentage germination for same size seed increased with later harvest (Austin & Longden, 1967). The spread of seed emergence and CV of seed weight decreased with delayed harvest (Gray & Steckel, 1980). A harvest delay of approximately one month decreased the time of seedling emergence by six days, reduced the spread of germination from 1.33 to 0.79 days (log data) and reduced the mean time to germination from 14.5 to 8.5 days for all umbels, with the same pattern for individual umbel orders (Gray, 1979). In the same study, for early harvested seeds, the embryo length from primary umbel seed was greater than that from the secondary umbels, but there was no difference at a later harvest date. Similarly, the CV of embryo length at early harvest was lower for primary than secondary umbels but the difference disappeared at a later harvest. The above examples show that delaying harvest generally improves seed quality, particularly for

seed from secondary umbels. This is because seed embryo growth and increase in seed weight occur at different times (Figure 2.1) (Gray & Steckel, 1983b).

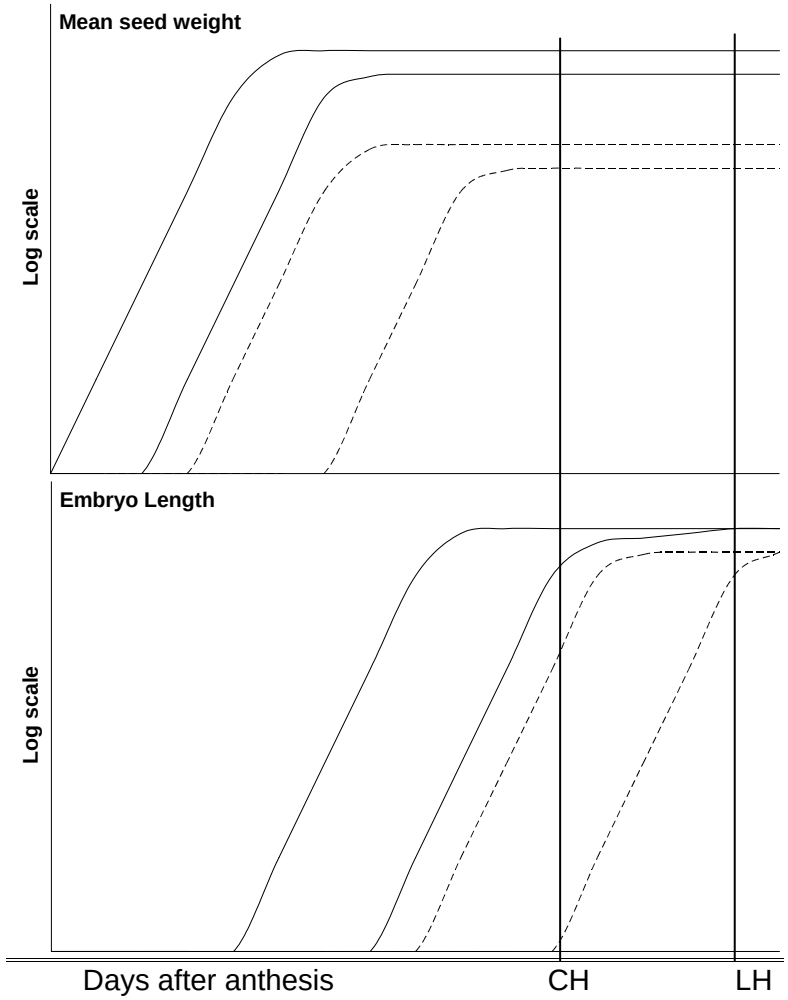

Figure 2.1. Schematic representation of increase in mean seed weight and embryo length for a population of seeds from the primary (———) and secondary (---------) umbels in which the start of seed and embryo growth of the individuals in the population is spread over a period of time, the extent of which is represented by the two lines. CH and LH represent a commercial and late harvest date, respectively. (After Gray & Steckel, 1983b).

During the initial part of seed formation most of the increase in seed weight is due to endosperm growth. In comparison, embryo development is delayed and continues when the endosperm has reached its maximum size. Also, due to the spread of flowering within each umbel order there is a wider range of both seed weight and embryo length while the seeds are maturing than when they have fully matured, as shown by the distance between the two lines for each umbel order in Figure 2.1 . CH is the time of a commercial harvest, i.e., when the seeds on the secondary umbels have turned brown but before the primary umbel seeds start to shatter. At this point seeds from both umbels have reached their maximum weight but the embryos in the secondary umbels are still developing. This means that the CV of embryo length for secondary umbel seeds will be significantly greater than had they been harvested at late harvest (LH)

where the embryos have matured. However, in a commercial situation, the crop would not be harvested at this time because the seeds on the primary umbels would have shattered from the plant and been lost, and climatic conditions would be less conducive for harvesting.

2.1.5. Seed vigour

Seed vigour (ISTA, 2006) is considered a particular problem in carrots because of significant variation in performance of seed lots over a range of field conditions (Currah, 1978; Perry, 1978a; Jacobsohn & Globerson, 1980).

While the concept of seed vigour is over 120 years old it is only recently that it has been clearly defined and accurate, repeatable tests have been devised to test for it. The origin of the concept of seed vigour is commonly ascribed to F. Nobbe in his 1876 book "*Handbuch der Samenkunde*" in which he used the German term "*Triebkraft*", which literally translates as 'driving force', to describe the phenomenon (Perry, 1978b; Copeland & McDonald, 2001). However, it was not until 1978 that the first international definition of vigour was accepted by The International Seed Testing Association (ISTA). The current definition is:

> 'Seed vigour is the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments'
>
> Seed vigour is not a single measurable property, but is a concept describing several characteristics associated with the following aspects of seed lot performance:
>
> i. Rate and uniformity of seed germination and seedling growth.
> ii. Emergence ability of seeds under unfavourable environmental conditions
> iii. Performance after storage, particularly the retention of the ability to germinate.
>
> A vigorous seed lot is one that is potentially able to perform well even under environmental conditions which are not optimal for the species (ISTA, 2006).

Despite seed vigour being defined 25 years ago it was only in 2000 that vigour tests were included in seed testing rules and only after strong opposition from the International Seed Trade Federation was addressed (Hampton, 1999).

Vigour tests are needed because different seed lots with similar high percentage germination can exhibit widely differing rates of emergence when planted in the field, especially if conditions are suboptimal (Copeland & McDonald, 2001). Farmers, particularly vegetable growers supplying markets with strict product grading

requirements, need to establish crops within a narrow range of plant densities. If the difference between field germination and laboratory germination varies among seed lots, growers cannot accurately predict plant densities. The vigour test aims to be a more sensitive test of seed quality than the germination test and to provide consistent ranking of the potential performance of seed lots (McDonald, 1980). This means that a vigour test should estimate field emergence more accurately than a germination test. However, a vigour test cannot predict actual field emergence, due to the wide range of other factors that determine field emergence. Vigour tests are also valuable for estimating how well seed will store and its likely performance after storage.

Seed vigour testing is based on the fact that seed deterioration (by which vigour is lost) and seed viability loss occur at different rates. In Figure 2.2, at time A seed viability and vigour are both high and similar. However, at time B, while seed viability has decreased only slightly compared to time A, vigour has decreased significantly.

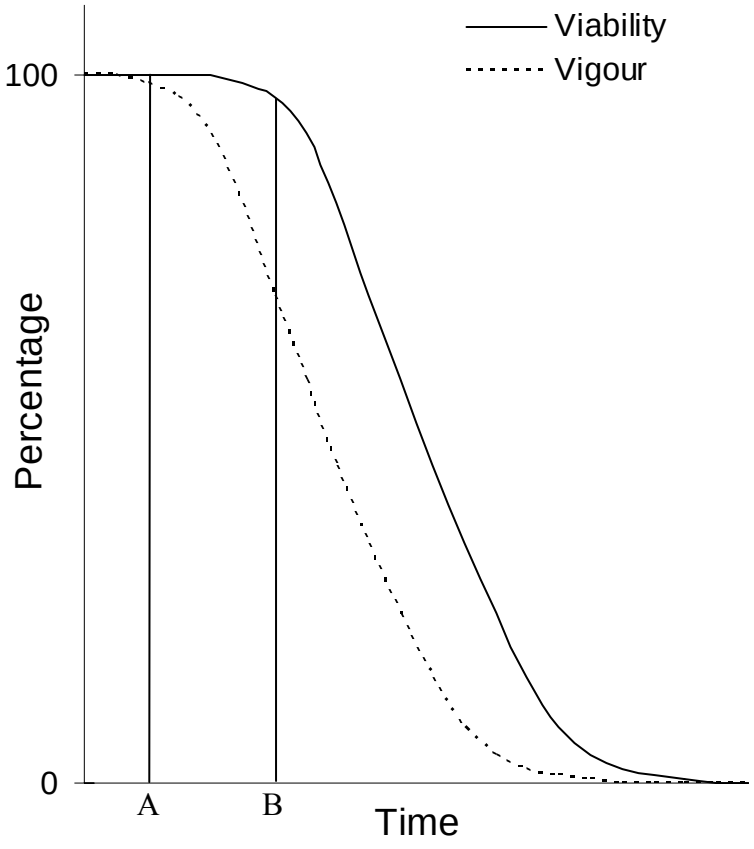

Figure 2.2. Relationship between seed viability and vigour over time. At time A seed viability and vigour are similar but at time B viability has only slightly decreased while vigour has significantly decreased (After Hampton, 1999).

The causes of poor seed vigour are many and include nutrition provided by, and position on the mother plant, environmental and climatic conditions during crop growth, stage of

maturity at harvest, post harvest treatments and genetic effects (Hampton, 1999), i.e., practically all aspects of crop production.

While carrots are widely regarded as having problems with vigour, little research has been conducted on how crop production techniques affect carrot seed vigour. Accelerated aging (AA) seed vigour tests on carrots grown at 20 and 45 plants m^{-2} under overhead or surface drip tape irrigation showed that germination for the primary, secondary and tertiary umbels across all treatments decreased from 84%, 84% and 64% respectively before accelerated ageing, to 43%, 31% and 8% after the AA test. Although no statistical analysis was done, the results strongly suggest a lower seed vigour for seed from the secondary and tertiary umbels (Thakan & Ramnut, 2002).

In 2001 the ISTA included vigour testing in its International Rules for Seed Testing (ISTA, 2002) for the first time with an accelerated aging (AA) test for soybean (*Glycine max* (L.) Merr.) and conductivity test for garden pea (*Pisum sativum* L.). The standardisation of the procedure for AA on soybean provides a well-tested methodology for the development of AA tests for other species, including carrots.

2.2. Management of *A. dauci, A. radicina* and *C. carotae*

2.2.1. Existing management options

There are very few options for control of *A. dauci*, *A. radicina* and *C. carotae* in organic carrot crops. The only fungicide that has been proven effective at controlling *A. dauci* on carrots, and that can be used in organic systems, is copper based (de Resende *et al.*, 1996; Ben-Noon *et al.*, 2001). However, the use of copper is restricted, with a maximum application rate of 3 kg ha^{-1} elemental copper per year by New Zealand/organic certification agencies and international organic standards, due to the negative side effects of such compounds on the environment (Bayley *et al.*, 1995; Reinecke & Reinecke, 1996; Anon., 2000; Anon., 2001). Furthermore, the total permissible application rate may be decreased in the future (Anon, 1991). There are no fungicides that can be used in organic systems that have been shown to be effective in controlling *A. radicina* and *C. carotae*. There is research that has demonstrated that copper and sulphur based fungicides can achieve some level of control against other *Alternaria* spp. (Archana *et al.*, 2001; Kushwaha *et al.*, 2001; Jyoti & Singh, 2002) and against *Cercospora* spp. (Dubey & Mishra, 1996; Yadav & Singh, 1999). As both sulphur and copper based fungicides are broad spectrum it is likely they will control all three pathogens to some degree.

In addition, synthetic chemical seed dressings and treatments that have helped to manage *A. dauci, A. radicina* and *C. carotae* in non-organic systems are also prohibited. Without an effective strategy to manage these pathogens in organic carrots, high levels of infection are likely in years when the weather is conducive to their spread. This is likely to lead to considerable yield losses and high levels of infested seed. This means that only F1 parent lines that have good resistance to disease can be grown in organic systems, which considerably limits the F1 crosses that can be produced. Therefore, an integrated management systems for *A. dauci*, *A. radicina* and *C. carotae* needs to be developed. For such a system to be successful, a thorough understanding of the biology of the pathogens is critical.

2.2.2. Biology epidemiology and etiology of *A. dauci* and *A. radicina*

A. dauci, and *A. radicina* are both non-obligate, parasitic, imperfect fungal pathogens of carrots with a world wide distribution (Ellis & Holliday, 1972; David, 1986; Pryor & Strandberg, 2001; Farrar *et al.*, 2004). Despite their wide distribution and potential to cause considerable economic loss, the amount of published information on the biology and etiology of the pathogens and the diseases they cause is relatively limited (Rotem, 1994).

2.2.2.1. A. dauci

A. dauci attacks carrot leaves, generally at the margins, stalks and flowers, forming small, dark, irregular, necrotic lesions, which are often surrounded by a chlorotic halo. Under optimal conditions these can coalesce and cover the entire leaf (Hooker, 1944; Strandberg, 1983). Older leaves are infested at a higher rate than younger leaves (Hooker, 1944). *A. dauci* also causes damping off of carrot seedlings, producing a continuous constricting lesion, tan brown in colour, extending from soil level upwards, often reaching and sometimes including the cotyledons (Maude, 1966). Conidia germinate between 4 to 37°C with a peak at 28°C. One hundred percent germination can occur within 3 h at 28°C. Maximum mycelial growth also occurs at 28°C with the temperature range being a maximum of 37.5°C and a minimum of less than 4°C (Hooker, 1944).

A RH of 96 to 100% or free surface water are required for conidiophore and conidia production and this only occurs on necrotic areas of infested carrot leaf at night . There is a correlation between increasing time that infested carrot leaves are wet overnight and increasing levels of conidia trapped the following day. Conidia were not found above

the crop during the night or when it was foggy or raining. While conidia were produced over a range of temperatures from 7 to 19°C, conidia production at 7°C was significantly reduced. Winds above 2 to 3 m sec^{-1} with RH less that 80% are required to dislodge conidia, while mechanical operations by farm machinery in the crop cause large numbers of conidia to be released with levels two to five times normal background levels (Strandberg, 1977). Other studies also showed that leaves had to be wet overnight with a temperature exceeding 12°C for there to be significant conidial release the following day (Langenberg *et al.*, 1977). This results in a clear diurnal cycle of conidia production at night and release during the day. Periods of continual high winds, cool temperatures or daytime leaf wetness resulted in limited conidia release. Conversely, during periods of continued strong winds sudden drops in conidia numbers were recorded due to the depletion of conidia.

In a series of laboratory studies (Strandberg, 1988) it was shown that the longer carrots were kept at 100% RH after artificial inoculation (up to 56 h) the greater the area of leaf infested. Wet periods longer than 56 h resulted in the death and collapse of entire leaves within 1 to 3 d of removal from the moisture chambers. Plants held in constant light for 24 h at 100% RH and 24°C had only 29% leaf area infested compared to 43% for plants kept in the dark. The number of lesions and damaged leaf area were closely related to number of conidia/inoculum levels applied. Older conidia were more infective than newly produced conidia. In follow up fieldwork infection levels were significantly higher for plants that had been infected and then kept in situations where leaves were wet at night compared with those where leaves were dry or RH less than 100%. However, infection levels dropped when night temperatures fell below 12°C. There was no significant difference in infection levels between plants that were four to eight weeks old, but infection levels were greatest on older leaves (Strandberg, 1988).

A. dauci can persist as both hyphae and conidia in the soil, on crop volunteers and on crop remains. In studies in carrot producing areas in California in the U.S.A., Pryor *et al.* (2002) found *A. dauci* on crop volunteers in all fields. Three to five months after infested carrot crops had been harvested samples of crop debri were taken from the surface, and from a 10 cm and 20 cm depth in the soil profile. All fields had infested crop debri, and of the debri collected, 69% was infested with *A. dauci* for all depths. When fresh infested leaf tissue was placed in nylon mesh bags in a range of field situations, *A. dauci* could remain viable on the samples placed in dry conditions on the soil surface for up to 12 months. However, for buried samples and samples placed on

the soil surface within irrigated crops, the time that they remained viable was reduced by a third. Greenhouse and laboratory tests of *A. dauci* survival showed that sporulation was abundant on infested carrot tissue stored in paper bags in ambient conditions for up to seven months and that sporulation was still moderate after 12 months. *A. dauci* also survived for 12 months on the surface or when buried in dry soil in pots in the glasshouse. In comparison, sporulation was considerably reduced for both surface placed and buried infested carrot leaf when pots were regularly watered, with sporulation ceasing after only one month (Pryor *et al.*, 2002). A similar pattern was found by researchers in Israel with the level of viable *A. dauci* on carrot tissue decreasing more rapidly when the carrot tissue was buried or wetted on a regular basis compared with only a 10% decrease in the number of viable *A. dauci* colonies on dry carrot leaf after 3 months (Netzer & Kenneth, 1969). In comparison, in the UK *A. dauci* would not infect carrots planted in soil in April in which infested carrot foliage had been incorporated the previous October (Maude, 1966). *A. dauci* infests carrot seeds both internally and externally (Netzer & Kenneth, 1969; ISTA, 2006).

Alternative hosts for *A. dauci* include wild carrot (*Daucus carota* L.), wild parsnip (*Pastinaca sativa* L.), fumitory (*Fumaria muralis* Sonder ex Koch), celery (*Apium graveolens* L.), *Daucus maximus* Desf., false fennel (*Ridolfia segetum* (L.) Moris), and *Torilis tenella* (Delile) Reichenb. fil., (Neergaard, 1945; Netzer & Kenneth, 1969; Soteros, 1979b).

2.2.2.2. A. radicina

A. radicina, in comparison to *A. dauci*, attacks both carrot leaves and roots. The pathogen can move from the leaves, down the petioles to infect the root and it can also infect the roots directly either through unbroken skin or wounds (Meier *et al.*, 1922; Lauritzen, 1926). Lesions on the leaves are tan-brown to dense black with a lighter area in the centre, and the root infection produces a dark to black mealy rot (Grogan & Snyder, 1952; Ellis & Holliday, 1972). *A. radicina* can also cause damping off of seedlings, producing a dense black coloured lesion over the entire length of the stem (Maude, 1966). The temperature range over which infection of roots and growth on agar takes place is -0.5 to 34°C with no infection or growth at 39°C, and a clear optimum temperature of 28°C (Lauritzen, 1926). *A. radicina* can overwinter in soil and crop debris. Maude (1966) found that it could survive at low levels overwinter in South England for up to seven months. In comparison Pryor *et al.* (1998) found that *A. radicina* populations in soil samples only decreased 54% over four years when stored

at ambient laboratory temperature and humidity, and that it was only the conidia that survived, while Maude and Shuring (1971) found that it could survive in soil in the absence of carrot cultivation for eight years. *A. radicina* infests carrot seeds both internally and externally (Soteros, 1979a).

Alternative hosts for *A. radicina* include celery (*Apium graveolens* L. var. *dulce* (P. Mill.) D.C.), parsnip (*Pastinaca sativa* L.), parsley (*Petroselinum crispum* (Miller) A.W. Hill), fennel (*Anethum graveolens* L.), dill (*Anethum graveolens* L.), celeriac (*Apium graveolens* L. var. *rapaceum* (Miller) Gaudin), *F. muralis* and caraway (*Carum carvi* L.), (Neergaard, 1945; Ellis & Holliday, 1972; Gindrat, 1979; Richardson, 1979; Soteros, 1979b; Coles, 2003). The list of alternative hosts for *A. dauci* and *A. radicina* are not considered definitive as the methodological validity of some of the earlier reports has been questioned (Farrar *et al.*, 2004)

2.2.2.3. Similarities between *A. dauci* and *A. radicina*

There are a considerable number of similarities between *A. dauci* and *A. radicina*, such as optimal temperature for growth and requirements of surface water for germination. Rotem (1994) in a review of the genus *Alternaria* noted that the pathogenic *Alternaria* spp. share four basic characteristics:

- All *Alternaria* are highly resistant to adverse weather;
- They develop in a wide range of temperatures and use the locally available source of moisture;
- They sporulate best on necrotic and dead tissue and produce a relatively small number of conidia, mainly at the end of the season;
- They are decisively affected by the age-conditioned susceptibility of host plants (Rotem, 1994).

The similarities between *A. dauci* and *A. radicina* and the considerable homogeneity of the characteristics of the pathogenic *Alternaria* spp. allow a general description of the biology and etiology of both pathogens, including characteristics that have not been studied for these pathogens, such as the effects of interrupted wet periods on infection and sporulation.

The pathogens are most damaging on seedlings and mature plants. Vigorous, vegetatively growing plants are less susceptible. The disease worsens with plant age, accelerating when reproductive growth occurs, and stressed plants are more susceptible. Light is generally required for conidiophore formation while conidia production is

enhanced by a period of darkness. Humidities of greater than 95% or surface water are required for the production of conidiophores and conidia; however, an interrupted wet period (IWP) may be needed between conidiophore and conidia production. A two-night cycle of sporulation has been observed for *A. dauci* (Rotem, 1994). IWPs are tolerated well by the fungi during both infection and conidia production. Sporulation mostly occurs on necrotic tissue with dry windy conditions needed for conidia dispersal. Physical disturbance of the crop during agricultural field operations can cause very large numbers of conidia to be released. Depending on temperature, sporulation can start within a day of infection and continue for about a month, with higher temperatures producing rapid sporulation and cooler temperatures giving more prolonged sporulation.

Free water or 100% humidity is essential for conidia germination and infection; optimum temperatures are about 28°C, with a maximum around 35°C and the minimum being unclear. Germination takes 1 to 3 h. While *A. radicina* and *A. dauci* can germinate and penetrate carrot tissue during interrupted wet periods, continual wet periods produce greater infection, and the longer that period, the higher the rate of infection.

The fungi are well adapted to survive in the absence of host plants, with both the mycelium and conidia being able to remain viable for years in dry conditions. However, the mycelium appears to need crop debris for survival, and the accelerated decomposition of such residue in moist conditions reduces the length of survival. Conidia can sit on leaves for many months before germinating with little effect on the number of infections. Overseasoning can also occur on alternative hosts.

Transmission through infested seeds and air dispersal are the main methods for infecting crops where infested soil or crop residue is not present. Air dispersal is facilitated by the large size of the conidia which enables them to float in the air, and the presence of melanin and their multicellular structure protects them against solar radiation. *Alternaria* conidia have been found many hundreds and even thousands of kilometres from potential sources of infection and also many kilometres high in the atmosphere, thought it is not recorded if they were pathogenic or saprophytic species (Rotem, 1994). Dispersal by rain splash is limited, and generally, dry windy conditions are required for conidia to be released from the conidiophores. There also tends to be an annual pattern of dispersal, with a peak in the autumn due to the limited infection of young crops in the spring, compared to much greater infection and sporulation on senescing crops and dead

leaves. There is also a clear diurnal dispersal pattern with a minimum at night and maximum at the warmest, driest and windiest part of the day, generally around noon.

2.2.3. Biology and etiology of *C. carotae*

C. carotae is a non-obligate, parasitic, fungal pathogen of carrot with a world wide distribution (Chupp, 1953). It infests both wild and cultivated carrots and the related species *Daucus gingidium* L. and *Daucus pusillus* Michx. (Thomas, 1943). *C. carotae* has also been shown to grow and sporulate when a suspension of conidia was placed on both sterilised and unsterilised soil (Thomas, 1943).

The fungus attacks all aerial parts of the carrot, producing round to elliptic lesions, starting pale tan and progressing through grey and brown to almost black. Lesions can appear on any part of the stalk and leaf but are often located along the edge of the leaflets. The lesions are surrounded by a diffuse chlorotic border, and can merge to affect the whole leaf, thus killing it. On the petioles and stems, lesions are linear and can girdle the tissue. The leaf tissue is penetrated via stomata or wounds; there is no evidence of direct penetration or appressoria (Thomas, 1943). Infection takes between 36 and 72 h.

C. carotae conidia germinate between 8 and 32°C with a maximum rate of germination at 24 to 28°C. Mycelium growth occurs between 8 and 37.5°C with maximum growth rates occurring at 28°C (Hooker, 1944; Carisse & Kushalappa, 1990, 1992; Carisse *et al.*, 1993). Relative humidity needs to exceed 84% or there needs to be free water on the leaf surface for infection to occur. The presence of water on the carrot leaf surface for a period of 6 h or more has been shown to significantly increase infection levels even if the leaf is dry for up to 36 h before becoming wet again (Carisse & Kushalappa, 1992). *C. carotae* has been shown to infest younger carrot leaves at significantly higher levels than older leaves, which is in contrast to *A. dauci* which attacks older leaves (Hooker, 1944; van Delden & Carisse, 1993). Sporulation on carrot leaves can start as soon as 48 h after inoculation in optimum conditions with maximum production achieved after 96 h. The rate of conidia production is increased by the presence of water on the leaves and/or a high RH (96%). The optimum temperature for conidia production is 28°C with a minimum of about 12°C and maximum of 32°C (Carisse *et al.*, 1993). *C. carotae* is externally seed borne (Thomas, 1943) but there are no records of it being found inside the seed. It also survives on dead carrot foliage up to nine months in field conditions (Thomas, 1943).

2.2.4. Similarities among *A. dauci, A. radicina* and *C. carotae*

There are a number of biological and etiological similarities among the three fungi. All are seedborne and infect carrot leaves only in the presence of very high humidities or surface water for a number of hours. They can infect, grow, and sporulate over a temperature range that is commonly found in areas suitable for carrot root and seed production. The time between infection and sporulation can be a matter of days, so the pathogens are polycyclic. This means that low levels of initial infection can result in epidemics if conditions for pathogen growth and sporulation are conducive. They can all persist on crop debri for a number of months in moist conditions and years in dry conditions. The conidia are spread by wind and could be moved a considerable distance from the inoculum source. These similarities give the opportunity for a single integrated management program for the three pathogens.

2.3. Integrated disease management for *A. dauci*, *A. radicina* and *C. carotae*

2.3.1. Resistant cultivars

A suggested integrated disease management system for *A. dauci*, *A. radicina* and *C. carotae* in organic carrot seed crops is based on the use of less susceptible cultivars, disinfection of seeds, the isolation of crops in space and time, the minimisation of other sources of cross-infection such as field workers and agricultural machinery, the optimisation of planting density and the use of biological controls and/or permitted fungicides. The rationale for this approach is based on the following:

Carrots vary in their susceptibility to the three pathogens with some cultivars having high levels of resistance to *A. dauci* and *A. radicina* while others are highly susceptible (Stein & Nothnagel, 1995; Dugdale *et al.*, 2000; Pryor *et al.*, 2000). No carrot cultivars have shown complete resistance to *C. carotae* though there are still significant variations in resistance levels among cultivars (Lebeda *et al.*, 1988). In F1 hybrid carrot production the parent lines are deliberately inbred and show considerable variation in their susceptibility to the pathogens, which may be different to the susceptibility of the F1 generation (James Smith, pers. comm., 2003)

2.3.2. Pathogen spread and control

Transmission of the pathogens via carrot seed is the main mechanism by which *Alternaria* spp. are introduced into previously disease-free areas (Maude & Humpherson-Jones, 1980; Herr & Lipps, 1982). Surveys of commercial carrot seed lots

in several countries have revealed a range of *A. dauci* and *A. radicina* infection levels within lots from 0.04% to 75%, with up to 22% of lots infested (Maude, 1966; Netzer & Kenneth, 1969; Soteros, 1979a) There is only one reported survey for *C. carotae* in commercial seed lots, and the pathogen was not detected (Grogan & Snyder, 1952). Exclusion or eradication of pathogens from carrot seeds is considered a fundamental disease management tactic (Walker, 1952; Fry, 1982; Sherf & MacNab, 1986).

Once the pathogens have been introduced into a farming area via infested seeds, alternative hosts, crop debris and overwintered crops are important means by which the pathogens survive to infect later crops (Thomas, 1943; Rotem, 1994). The pathogens are able to survive for more than a year in a field situation following infested crops, alternative hosts have the ability to maintain the pathogens in an area, and conidia are able to be dispersed considerable distances from current crops; therefore, there is a need to isolate crops in space and time from previous and current carrot crops (Thomas, 1943; Maude & Shuring, 1971; Pryor *et al.*, 1998; Pryor *et al.*, 2002). Rotations and crop isolation are already standard techniques within both organic agriculture and vegetable seed production as a means of managing pests and diseases, and minimising pollen contamination between different cultivars of the same crop (Kranz & Rotem, 1988; Lampkin, 1994; George, 1999).

Field workers and agricultural equipment have been shown to be vectors of *Alternaria* and other fungal pathogens (Walker, 1952; Rotem, 1994; Kristensen & Borgen, 2001). Potentially both conidia and mycelium could be spread, for example on field worker's clothes and on crop debris and soil on machinery. Only very low levels of infection (e.g., 1%), are required for disease outbreaks to occur in crops (Jackson *et al.*, 1987). Therefore, while the amount of inoculum transmitted may be small, the potential for succeeding generations of the pathogens to build up to epidemic proportions is considerable (Rotem, 1994).

Differing plant populations have been shown to alter the microclimate within crop canopies (e.g., Tompkins *et al.*, 1993; Fernandez *et al.*, 2002). *A. dauci*, *A. radicina* and *C. carotae* all need high humidities or wet leaves for several hours to infect and sporulate. Increasing plant densities increase the humidity and length of wet periods within the crop canopy and therefore increase the risk of disease outbreaks. However, higher carrot populations can have benefits in terms of increased quantity and quality of seed, so there is a conflict between lower populations for disease management and higher populations for increased yield and quality.

Despite taking measures to minimise the infection of crops, a disease risk is still likely to exist. A need to manage the pathogens if they do occur in the field is required. The use of copper is restricted to 3 kg active ingredient ha year^{-1} and while sulphur can be used in greater amounts, its effectiveness is unknown. Therefore, there is a need to identify new control materials. One area that may provide such materials is the increasing number of biological control agents for plant fungal pathogens, some of which may be effective against *A. dauci*, *A. radicina* and *C. carotae* (Upadhyay *et al.*, 2000).

2.3.3. Potential biological control agents for *A. dauci*, *A. radicina* and *C. carotae*

A limited number of potential biological control agents for *A. dauci* and *A. radicina* and other *Alternaria* spp. have been identified from the literature. Effective Microorganisms (EM) a water based mixed culture of microorganisms, including yeasts, lactic acid bacteria, photosynthetic bacteria, *Actinomycetes*, fermenting fungi, *Aspergillus* spp. and *Penicillium* spp. (Higa, 1994) have shown limited ability to suppress *Alternaria* spp. in a double-layer plate test with a 1% solution of EM producing a 25% suppression and a 5% solution giving 38% suppression (Castro *et al.*, 1993b). Two antifungal antibiotics, iturin D and iturin E, isolated from an iturin A producing strain of *Bacillus subtilis* (Ehrenberg) Cohn demonstrated strong antifungal activity against *A radicina* (Besson & Michel, 1987). Soaking carrot seed in a suspension of *B. subtilis* str. T99 improved carrot seed germination and seedling health in the presence of *A. radicina* in the laboratory (Hentschel, 1991). *Trichoderma viride* (Pers.) showed strong antagonism to *A. radicina* on stored carrots (Sesan, 1990). *Agrobacterium radiobacter* (Beijerinck and van Delden) (Young *et al.*) has been shown to strongly inhibit the growth of *A. alternata* (Fries : Fries) von Keissler in vitro (Catska, 1989). No reports of tests of potential biological control agents against *C. carotae* have been found.

In New Zealand the above organisms are available as commercial BCA: *B. subtilis* is sold as Serenade® (AgraQuest Ltd., Davis, California, USA), *T. viride*, as Trichoflow® and related products (Agrimm Technologies Ltd., Christchurch, New Zealand) *A. radiobacter* as Dygall® (AgBioResearch Ltd., Richmond, New Zealand). *A. radiobacter* has been renamed as *Rhizobium rhizogenes* (Walton, 2001; O'Connor, 2002).

There are limited indications that EM has potential as a broad spectrum bio-fungicide (Castro *et al.*, 1993a; Jonglaekha *et al.*, 1993) while *B. subtilis*, *T. viride*, and

A. radiobacter all have demonstrated potential as bio-fungicides that can control a number of different diseases (Krebs *et al.*, 1998; Butt *et al.*, 2001). Therefore, there is potential for the biocontrol agents listed above to control *A. dauci*, *A. radicina* and *C. carotae* on carrots.

Biological control microorganisms have a number of modes of action which can be categorised as; antagonism, antibiosis, competition, mycoparasitism and induced resistance (Mukhopadhyay, 1994; Lo, 1998). To date the majority of biological control research has focused on using one biocontrol agent to combat one disease (Bellows *et al.*, 1999). Mixtures of organisms with different modes of action may have the potential to enhance the spectrum of activity and level of control but at present there is limited knowledge and understanding of interaction of such mixtures. As two of the above biocontrol agents are bacteria, one is a fungus, and their modes of control differ, there may be a possibility for synergistic interactions that could enhance disease control, providing that the biocontrol agents are not antagonistic to one another.

2.3.4. Control of seed borne *A. dauci*, *A. radicina* and *C. carotae* by heat

Hot water treatment of carrot seed has been shown to be effective at significantly reducing *A. dauci* and *A. radicina* seed infestation levels with limited reduction of seed germination. Germination was unaffected when seeds were treated at 55°C for 20 min, but 8 min or more at 60°C, and more than 20 min at 55°C did reduce germination (Strandberg & White, 1989). In a test of 25 genetically diverse cultivars using 15 min at 50°C, emergence was reduced in 76% of the cultivars (range 0.1 to 13.2%) but only a third of the reductions were significant (Strandberg & White, 1989). Treatment at 50°C for 20 min. eradicated *A. radicina* from infested carrot seed with minimal reduction in germination, but treating the seeds for 30 min did significantly reduce germination (Pryor *et al.*, 1994). In other studies treatment at 54°C for 20 min eradicated *A. dauci* without adversely affecting germination, emergence, yield or storage quality, while treatment at 59°C for 15 min did reduce germination (Hermansen *et al.*, 1999). In contrast, *A. dauci* was not completely eliminated at 20 and 30 min at 55°C but at 40 min it was. The percentage of infested seeds at 20 min was approx. 2%, and at 30 min was approximately 1%, which was similar to the level of control gained with Iprodione (Strandberg & White, 1989; Hermansen *et al.*, 1999).

As an alternative to hot water treatments dry heat treatment of carrot seed at 70°C for 0, 3, 6, 9, 12 and 15 days was studied. Dry heat at 70° for 15 d eliminated unnamed seed

borne fungi infesting high quality carrot seed without reducing germination or vigour. However, poor quality and older seeds were negatively affected by heat treatments (Trigo *et al.*, 1998).

No literature has been found describing any heat treatment, nor other methods that would be acceptable under organic production rules, for eliminating *C. carotae* from carrot seed.

2.3.4.1. Variation between seed lots due to the condition of the seed

While at first the above results appear promising, there are a number of differences between the results and some unaddressed issues.

The hot water and hot air treatments, described above, are similar to the controlled deterioration and accelerated ageing vigour tests (Matthews, 1980; TeKrony, 1995). The vigour tests treat partially imbibed seeds for a period of one to three days at temperatures around 45°C, while the hot water treatments treat initially dry seeds at the higher temperatures of 50 to 55°C for the shorter periods of 30 to 60 min, and hot air treats dry seeds at 70°C for up to 15 days, i.e., all methods heat seeds for a specified period of time. It is, therefore, not unreasonable to expect seeds to react, in terms of reduced germination, in a similar, though quantitatively different way, to all methods.

Vigour tests are designed to be able to differentiate among seed lots with similar high percentage germination when tested under controlled conditions but which have significantly different seedling emergence when grown in the field (Copeland & McDonald, 2001). There are a number of factors that can cause seed with the same percentage germination to perform differently in vigour tests including, cultivar (including hybrid vigour), mechanical damage, seed chemical composition and physiological age of the seed (Copeland & McDonald, 2001). It is therefore likely that the seed attributes that are tested by vigour tests will also affect the seeds' response to heat treatments, i.e., seeds of high vigour should be less affected by a heat treatment than seeds of low vigour.

There is some limited evidence for this hypothesis in the literature. High quality carrot seed was shown to be unaffected by dry heat treatment while poor quality seed was (Trigo *et al.*, 1998). Fresh lettuce (*Lactuca sativa* L.) seed was found to be less susceptible to heat damage than old seed, even when the old seed was of high vigour (Drew & Brocklehurst, 1985). Laboratory germination of soybean was unaffected by

heat treatment, but emergence in the glasshouse decreased for seeds over one year old while seeds younger than one year were unaffected (Pyndji *et al.*, 1987).

This is a potentially significant issue for the widespread application of heat treatments. Ideally, the effect of a heat treatment on the seed of a given species would be the same for all seed lots, and the effect should be able to be determined through empirical tests, as was attempted in the research described above (Strandberg & White, 1989; Pryor *et al.*, 1994; Hermansen *et al.*, 1999). However, if seed vigour alters how seeds respond to heat treatment this may not be possible, because treatment may cause a significant decrease in germination for one seed lot but not another. Whether such variation between seed lots will be problematic or not may depend on the difference between the temperature, time and other conditions required to eradicate the pathogen and the conditions that adversely affect the seed. For crop species where the conditions required to eradicate the pathogen(s) are significantly lower than the conditions that cause a reduction in percentage germination because of poor seed vigour, then the difference in response between seed lots is likely to be small. Where the conditions required for successful pathogen eradication and a reduction in germination because of poor seed vigour are similar, then the effects of heat treatments among seed lots is likely to be variable.

2.3.4.2. The effect of seed moisture on the response of seeds to heat

In vigour tests the seed moisture content must be within defined levels and in the controlled deterioration test (ISTA, 2006) it must be accurate to three decimal places because the results of the test are very sensitive to moisture content. In the heat treatment studies (discussed above) no account was taken of seed moisture content, so it is possible that differences in the initial moisture content may be responsible for some of the differences between the studies.

The survival of *A. dauci*, *A. radicina* and *C. carotae* has been shown to vary considerably with the RH or moisture level of their environment (Thomas, 1943; Maude, 1966; Netzer & Kenneth, 1969; Maude & Shuring, 1971; Pryor *et al.*, 1998; Pryor *et al.*, 2002). It is, therefore, possible that the length of time that they can survive raised temperatures may depend on the amount of moisture present in the seed.

Seed moisture levels may also affect the seeds' response to heat treatment, as well as the pathogens, as demonstrated by seed vigour testing. There is also a considerable difference in treatment length between hot water and dry heat treatments, with higher temperatures often being used with dry heat (for example, Zeigler *et al.*, 1987; Tenente

et al., 1999; Mendes *et al.*, 2001). Seed moisture level would appear to be a critical factor in determining seeds' tolerance to raised temperatures.

For hot water, or other treatments in which the seed moisture content may be raised during treatment, the effect of different initial seed moisture contents on the results may not be straightforward. At very low moisture content, imbibition damage (Powell, 1986) may occur. At higher moisture content there may be no imbibition damage but the seed is still dry enough to minimise the effect of the heat. However, as moisture content increases the seeds may become more susceptible to heat. Hot water treatments may affect the storage capabilities of seed (Strandberg & White, 1989), which is substantiated by the requirement of vigour tested seeds to be germinated immediately (Copeland & McDonald, 2001).

2.4. Overwintered weed control by thermal weeders

2.4.1. Selective thermal weeding

Organic carrot seed production by the seed-to-seed method presents new weed control issues. The density of a carrot seed crop is much lower (20 plants m^{-2}) than root crops (100 to 500 plants m^{-2}), and the February sowing date results in carrots being 10 to 20 cm high when growth stops in winter, which means that crop is a weak weed competitor. In summer, this would not be a problem as the dry conditions allow the use of weeding machinery. However, in winter, fields are frequently so wet that they prohibit machinery access, and even if access were possible, the wet cold conditions would considerably reduce the effectiveness of hoeing equipment (Parish, 1990). By spring, when soil and weather conditions have improved sufficiently to allow access, weeds have reached such a size that existing intrarow weeding equipment, e.g., torsion and finger weeders (Welsh *et al.*, 2002), is unable to kill intrarow weeds. Alternative methods to control established intrarow weeds without resorting to expensive hand weeding are required.

One technique that has potential for controlling weeds in the spring is thermal weeding. Thermal weeders are used predominately to kill small weeds in the stale seedbed technique (Merfield, 2002) where the seedbed is created one or more weeks prior to crop sowing, which means that the majority of the weeds emerge before the crop. Weed seeds that germinate before the crop emerges can be killed with a thermal weeder without disturbing the seedbed. Thermal weeders can also be used after crop emergence if the crop has greater tolerance to thermal treatment than the weeds. Examples of such

crops are onions (*Allium cepa* L.) from sets and seed (Berge & Holmøy, 1998; Meyer *et al.*, 2002), some herbs (Bertram, 2003), cabbages (*Brassica oleracea* L.)(Holmøy & Storeheier, 1993), cereals (Juroszek *et al.*, 2002), sunflowers (*Helianthus annuus* L.), maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) (Peruzzi & Raffaelli, 1999).

Thermal weeding after crop emergence involves two main approaches. First, both crop and weed foliage are destroyed; however, the crop's apical meristem is protected by either being under ground, e.g., onions, or in a rosette of leaf bases, e.g., carrots. Second, the thermal treatment is targeted at the intrarow area at the crop stem's base. The latter approach is mostly used when crops are larger or for species that have a stem that can tolerate moderate heat exposure, e.g., cabbage and perennial crops such as grapes (*Vitis vinifera* L.). The first approach can be used on monocotyledonous crops shortly after crop emergence, e.g., at the three true leaf stage, when the weeds are also small and can be easily killed. It can be used only in dicotyledonous crops at later growth stages when they have developed sufficient foliage to protect the apical meristem. However, research studying the effect of thermal weeding on different weed growth stages shows that for a fixed amount of energy weed survival follows a logistic curve with high mortality at smaller growth stages then an exponential increase in the number of surviving weeds and then a plateaux when the weeds are sufficiently large and none are killed. The amount of energy required to kill progressively larger weeds also increases exponentially (Ascard, 1994, 1995; Vanhala & Rahkonen, 1996; Peruzzi *et al.*, 1998; Heisel *et al.*, 2001; Sartorato *et al.*, 2006). Most crop weeds germinate in response to cultivation, which means that when the crop is old enough to withstand thermal treatment the weeds will also be larger and therefore more difficult to kill, making thermal weeding at larger crop growth stages of more limited use. The exception is for perennial crops, where weed seedlings are emerging from the bare soil in the crop row. For thermal control of overwintered weeds in the spring to be successful requires that the weeds will still be susceptible to thermal control, which is less likely if they are large and well established. However, even if only a proportion of weeds can be controlled, it may still be a cost effective technique considering the high cost of hand weeding.

Removal of crop foliage by thermal treatments may also have other positive and negative effects. It is likely that removing the foliage will cause a check in the crop growth due to the loss of photosynthetic leaf tissue, and the need to draw on nutrient and energy reserves within the root to produce new leaves. It is not clear how much of

a set back this will cause the plants, but some research indicates that partial loss of foliage may not be detrimental for small carrots. In two years of trials with the cv. Local Sel, at plant densities of 11, 5 and 2.7 plants m^{-2}, with none, one-third or two-thirds cut from the bottom of the steckling's root, and tops trimmed to one-third or two-thirds of full size or left intact, the highest average seed yield of 1,104 t ha^{-1} was obtained from stecklings spaced at 11 plants m^{-2}, using intact roots and with foliage cut to two-thirds of full size (Lal & Pandey, 1986). In addition, for root to seed production where carrots have to be stored indoors, the tops are often removed (Gray, 1979). These two examples indicate that partial foliage loss can be beneficial and that foliage removal is not catastrophic for the steckling. In addition, in both of these examples the stecklings are lifted from the soil, breaking off the taproot and lateral feeding roots, so removing the steckling's ability to take up water until the carrots are replanted and have regrown feeding roots. It is, therefore, reasonable to assume that overwintered carrots should be able to tolerate complete loss of foliage without killing the plants. It is however, unknown how far into the growing season whole-crop thermal weeding could be used, especially when carrot crops change from vegetative to reproductive growth.

2.4.2. Control of *A. dauci*, *A. radicina* and *C. carotae* through thermal weeding techniques

A secondary effect of thermal weed control and crop foliage destruction is the potential for disease control. *A. dauci*, *A. radicina* and *C. carotae* are all leaf and petiole infesting pathogens, although *A. radicina* can also infest the root (Meier *et al.*, 1922; Lauritzen, 1926). It is possible that the destruction of the foliage, surrounding weeds and heating of the soil surface during thermal weeding could potentially eliminate or considerably reduce pathogen levels in the crop. Alternatively, if the pathogens are not completely eliminated the damage to the plant from thermal treatment may enable the pathogens to more readily attack regrowing foliage. Elimination or a large reduction in the level of pathogens by thermal means in a field situation appears to be a novel approach, as no reports have been found in the literature.

2.5. Summary

The three key areas of research identified for organic carrot seed production in the Canterbury region of New Zealand are:

- The effect of crop density on seed yield and quality, including pathogen infestation of seed;

- The thermal management in spring of overwintered intrarow weeds in steckling crops;
- The management of fungal pathogens by an integrated approach including heat treatment of seeds, thermal treatment of growing crops in the spring and identification of potential biological control agents and their cross compatibility.

Chapter 3. Crop density

3.1. Introduction

To study the effect of plant density on carrots a radial trial design (Nelder, 1962; Bleasdale & Salter, 1982) was used. This approach has the advantage over a block design of requiring lower numbers of plants and less space as each arc acts as a guard row for its neighbours. The densities of 5, 11, 20, 55 and 82 plants m^{-2} were chosen to include the 20 plants m^{-2} used by industry and the higher densities used by other researchers.

3.2. Methods

A randomised complete split plot design was used, employing a full-circle radial design to determine the effect of carrot crop density on yields and seed quality. The main plot was densities of 5, 11, 20, 55 and 82 plants m^{-2} and the sub-plot was two Bejo Zaden B.V. (Warmenhuizen, The Netherlands) F1 hybrid seed parent cultivars A680339 and A680039. A680339 (referred to as cultivar A) is considered to produce more lower order umbels and to be more prostrate in contrast with A680039 (referred to as cultivar B), which is considered to produce considerably fewer lower order umbels and to have a more upright growth habit (James Smith, pers. comm. 2002). They, therefore, provided two contrasting growth habits and may react differently to the different cropping densities. Two circles were planted next to each other at the Biological Husbandry Unit, Lincoln University, New Zealand (43°39'005''S, 172°27'250''E) on a Wakanui silt loam. Each circle consisted of 60 radii and 17 arcs[1] resulting in densities of 100, 82, 67, 55, 45, 37, 30, 25, 20, 17, 14, 11, 9, 7, 6, 5 and 4 plants m^{-2} The inner- and outermost arcs were guard 'rows'. The sixty radii were divided into alternating sections of six radii of seed parent A, two pollen parent radii (C680459), six radii of seed parent B and a further two radii of the pollen parent. As the alternating pattern of two pollen and six seed parents did not divide exactly into the 60 radii, one seed parent section only contained four radii and was not included in the analysis. Each circle had three sections with one seed parent and four sections with the other, and the two circles alternated the number of sections for the seed parents so that a total of six replicates for both seed parents was obtained. The outside radii of each seed parent that grew next to the pollen parent were guard rows. All three cultivars are considered to have good resistance to *A. dauci*, *A. radicina* and *C. carotae* (James Smith pers. comm. 2004).

[1] Distance of arcs from the circle centre: 1.00, 1.11, 1.22, 1.35, 1.49, 1.65, 1.82, 2.01, 2.22, 2.46, 2.72, 3.00, 3.32, 3.66, 4.05, 4.47 and 4.94 m

The site had been under mixed pasture for the previous five years, and it was prepared by ploughing and rotary hoeing, with the final tilth manually prepared. No fertilisers were used as it was considered there should be sufficient nutrients, particularly nitrogen, after the long term pasture. From 23 January to 4 March 2004, plants were grown in VS-508 Paperpots (Lännen Tehtaat Plc., Säkylä, Finland) in a BioGro (Anon., 2001) certified fish waste and tree bark compost and kept in an unheated glasshouse. The plants were then transplanted into the field, where walking boards were used to minimise soil compaction. The two circles were surrounded by a fence, at least three meters distant from the circle edges, made from shade cloth 1.2 m high, to keep out vertebrate pests and reduce *Psila rosae* (F.) ingress. The entire trial area was manually kept weed free. Operations within the crop, e.g., weeding, were performed from a mobile platform situated over the crop to avoid compaction and plant damage. Pollen parents were not removed after flowering, as is standard industry practice, because they were required to maintain crop density. Two hives of bees were situated 30 m from the site and were considered sufficient to ensure pollination. The trial was irrigated by T-Tape (T-Systems International, Inc., San Diego, California, USA) spaced 30 cm apart with irrigation water applied when the soil was dry to a depth of approximately 5 cm to bring the soil back to field capacity.

The plants were hand harvested on 26 March 2005 when the second level umbels had turned brown. From each sector and density, four plants were harvested and dried indoors for two weeks. Umbels were removed with each umbel level collected separately. The umbels were threshed in a Wintersteiger LD 350 thresher (Wintersteiger AG., Austria) using a 2.6 × 7.5 mm screen. The seed was graded using a Westrup seed cleaner (Westrup A/S, Slagelse, Denmark, model LA-LST): the scalping screen was 3.0 mm round, the top screen 1.75 mm slotted and the bottom screen 0.7 mm slotted with a shaker motor speed of 400 rpm. The fan settings as per the markings on the controls were pre-aspiration gate 1, filter valve 1.5, fan outlet valve 10. The seed was then passed through the indented drum section of the cleaner using a 3.0 mm indent.

For each umbel level of each seed parent cultivar the number of umbels was recorded and yield, thousand seed weight (TSW), germination and seed vigour (ISTA, 2005) with the accelerated ageing (AA) test (41°C for 72 h) used as the vigour test were determined. Within 15 min of removal from the AA chamber seed was subjected to a standard germination test (ISTA, 2005) the results of which are presented as the vigour result. The height of each plant from the top of the root to the highest umbel was

recorded and seed from the secondary umbel level of cultivar (cv.) B was tested for the presence of *A. dauci* and *A. radicina* according to the methods of ISTA (2006) using moistened blotters in Petri dishes.

3.2.1. Statistical analysis

A number of results required transformation due to unequal variances. Where this was required, the untransformed means are presented along with the transformed means and the $LSD_{0.05}$ for the transformed data. The type of transformation is described in the results section. For the analysis of germination and TSW, there were too many missing values to use ANOVA because there was insufficient seed in some samples to conduct tests so the GenStat version 8.2.0 REML function was used. This is based on the residual maximum likelihood method to analyse linear mixed models and estimation of variance components. Having insufficient seed in some samples is unavoidable in this experimental design, because as density increases the amount of seed from lower order umbels decreases and for the tertiary umbel level completely ceases. For both ANOVA and REML analysis, each umbel level was analysed separately, so statistical comparison could not be made between umbel levels.

A key aim of this trial was to compare the performance of the two cultivars at a range of densities. Usually, in a two factorial design, the means for both factors and their interaction are presented, if statistically significant. In this experiment, the density means are the product of the two cultivars, which are often quite dissimilar. For example, at a density of 82 plants m^{-2}, the primary umbel of cv. A had a yield of 155 g m^{-2} and B 10g m^{-2}, producing a mean of 83 g m^{-2}. This average has little biological meaning due to it having only two, dissimilar components. Therefore, as a key aim of this trial was to compare cultivars, not densities, the density means are not presented; instead the means of cultivar and cultivar × density interactions are presented, even if the interaction was not significant, as this allows the contribution of each cultivar to be shown. However, where there was a statistically significant result for density this is noted. The means of each density can be produced from the interaction data by calculating the average of the two cultivar means for a given density.

Linear, quadratic and cubic curves were fitted to the density factor using the GenStat V8.2.0 'POL' function. This produces two analyses: First, it indicates the shape of the density curve of the mean of the two cultivars. The p value for the linear fit indicates if there is a significant linear trend through the data, which is equivalent to a non-zero slope in a linear regression. The p value for the cubic fit indicates whether the slope is

constant, i.e., if the rate of change, changes over density. The p value for the sigmoidal fit indicates that the curve is more complex and the response may be difficult to describe. Second, it allows the density curves of the two cultivars to be compared to see if they differ significantly at the linear, quadratic and sigmoidal levels. For example, one cultivar may have a linear response to increasing density, while the other may show a sigmoidal response. However, as discussed above, in this study the mean of the two cultivars was of limited value. Therefore, for the curve fitting, if there was no statistical difference between the curves of the two cultivars, the similarity and the results for the shape of the mean density curve are presented. Where the density curves of the two cultivars differed statistically, only the comparison of the density curves of the two cultivars are presented, not the analysis of the mean density curve.

3.3. Results

In the tables of results the cultivars are referred to as A and B. The umbel levels are signified by U1 to U4 with U1 = primary umbel, U2 = secondary umbel, U3 = tertiary umbel, and U4 = quaternary umbel.

The split-plot design produces two LSD values for the cultivar × density interaction, one for comparing among plant densities (e.g., comparing the number of umbels for cv. A at different density levels), the other for comparing between the cultivars (e.g., comparing the number of umbels of cv. A and B at one density level). LSD values presented with the cultivar × density interaction data are labelled D-$LSD_{0.05}$ for comparison among densities and C-$LSD_{0.05}$ for comparison between cultivars.

3.3.1. Plant height

Plant height data were normally distributed and as the natural log transformation gave the same results as untransformed data, only untransformed results are presented. There was a significant effect ($p<0.001$) for both cultivar and density, with height increasing with increasing density, and a mean of 100 cm for cv. A and 135 cm for B ($LSD_{0.05}$ 10.50) (Figure 3.1). The interaction of density and cultivar was not significant ($p=0.374$).

There was no significant ($p>0.1$) difference between the density curves of the two cultivars, with both reacting very similarly to the changing densities (Figure 3.1). There was a significant fit of the mean density curve to linear ($p<0.001$) and quadratic curves ($p=0.008$), with there being little change in height at the two lowest densities, then a

rapid increase up to 20 plants m^{-2}, followed by a much slower linear increase from 20 to 80 plants m^{-2} producing a sigmoidal curve (Figure 3.1).

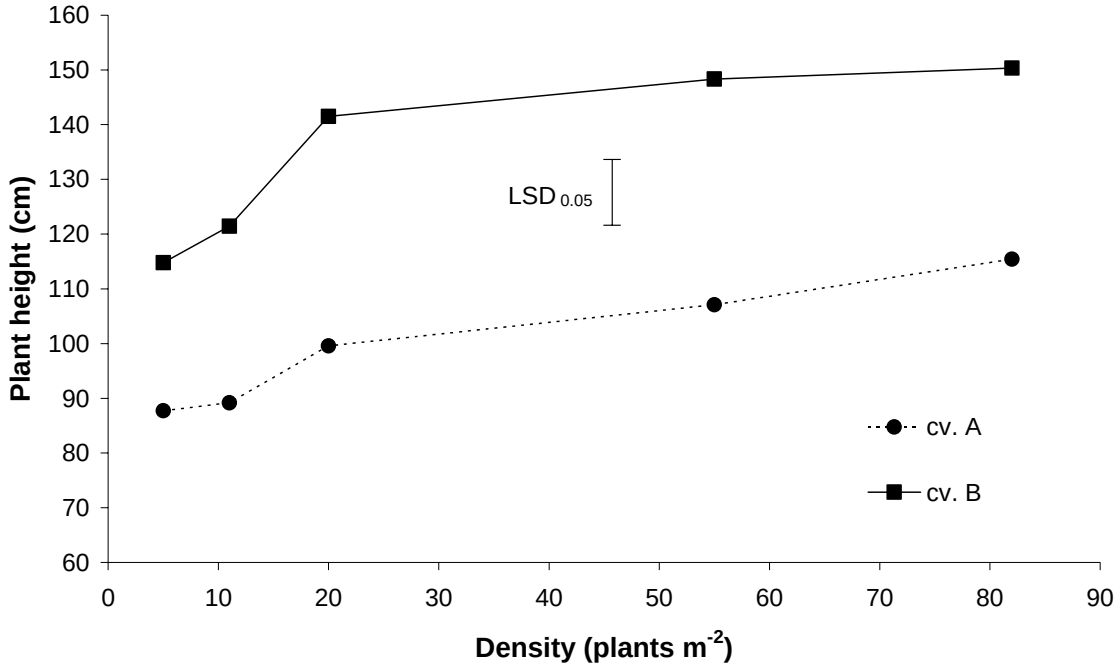

Figure 3.1. Effect of plant density (plants m^{-2}) on the height of two carrot F1 seed parent cultivars (A and B).

3.3.2. Umbel numbers

All plants retained their single primary umbel, so these were excluded from the analysis. Data were normally distributed, and as the square root transformation did not alter the results only untransformed data are presented. There was no significant difference in umbel numbers between cultivars at the secondary (p=0.598) or tertiary umbel levels (p=0.879) with the number of umbels being almost identical for the two cultivars (Table 3.1). However, there was a statistical difference (p=0.012) and a considerable biological difference at the quaternary umbel level (Table 3.1).

Table 3.1. Average number of umbels, at each umbel level, for two F1 carrot seed parent cultivars (A and B) values are means of five density levels.

		Mean	$LSD_{0.05}$
U2	**A**	8.8	1.68
	B	8.3	
U3	**A**	12.7	3.76
	B	12.5	
U4	**A**	5.6	2.52
	B	1.8	

There was a significant difference (p<0.001) among densities for all umbel levels with umbel numbers decreasing with increasing density (Table 3.2 and Figure 3.2). However, the interaction was not significant (U2 p=0.955, U3 p=0.696, U4 p=0.636).

Table 3.2. Effect of five plant densities (plants m^{-2}) on the number of umbels at each umbel level (excluding the primary umbel) of two F1 carrot seed parent cultivars (A and B).

		Density (plants m^{-2})						
		5	**11**	**20**	**55**	**82**	**C-LSD$_{0.05}$**	**D-LSD$_{0.05}$**
U2	**A**	12.8	11.0	8.7	5.1	5.2	2.24	2.0
	B	12.5	10.8	8.8	4.5	4.3		
U3	**A**	26.0	17.1	10.5	5.3	4.5	6.16	6.04
	B	27.3	20.0	9.2	3.8	2.0		
U4	**A**	13.5	6.3	3.2	2.5	2.5	5.51	5.77
	B	5.7	3.2	0.0	0.2	0.0		

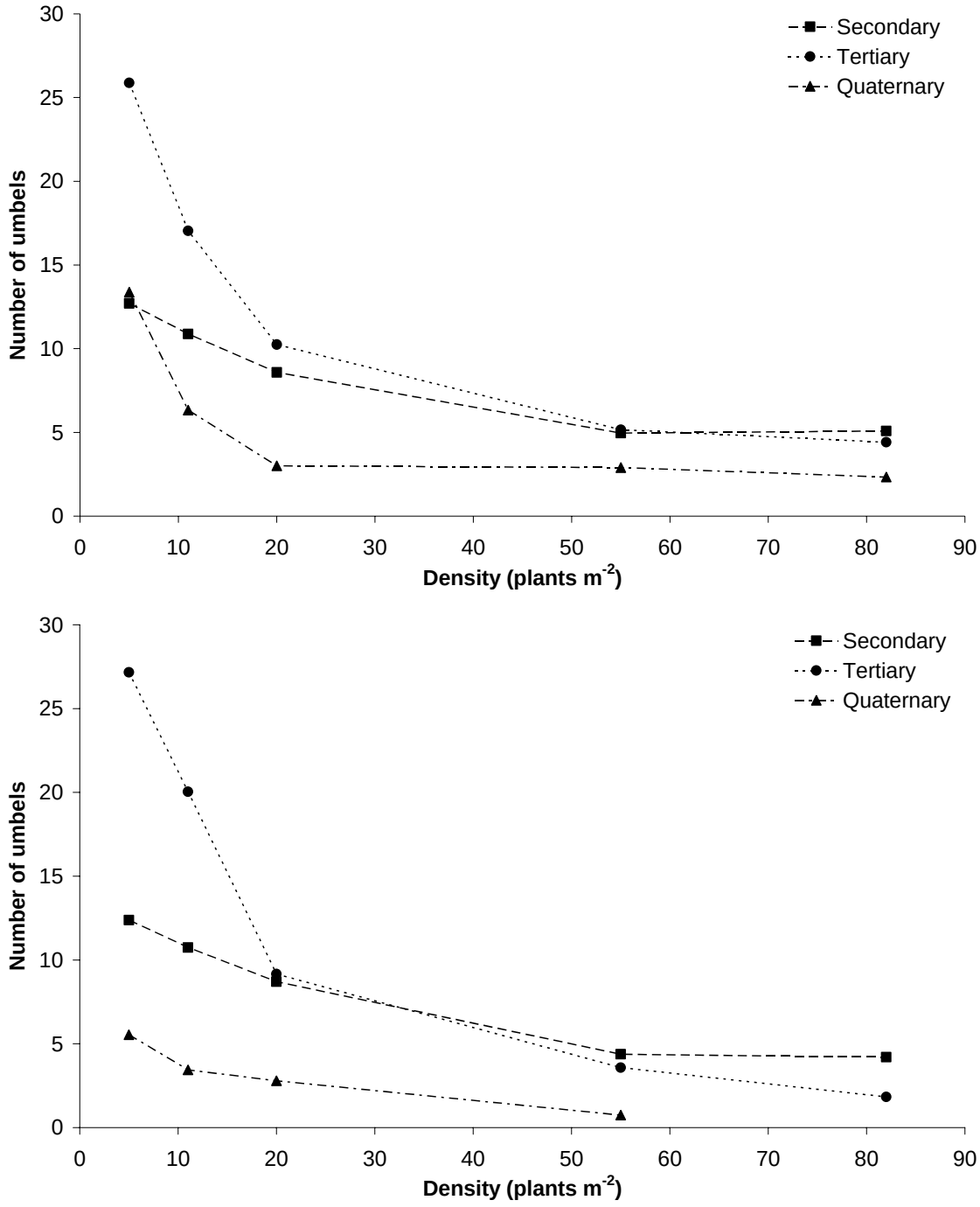

Figure 3.2. Effect of plant density (plants m^{-2}) on the numbers of lower order umbels of two carrot F1 seed parent cultivars (cv. A top, B bottom).

There was no significant difference (p>0.2) between the density curves of each cultivar for any umbel level. The mean density curve for the secondary umbel level was significant for the linear and quadratic fitted curves (p<0.001) but not for the cubic curve (p=0.612) (Figure 3.2). For the tertiary umbel level, the mean density curve was significant (p<0.001) for all three fitted curves with the number of umbels decreasing at a slower rate as density increased (Figure 3.2). The mean density curve for the quaternary umbel level was significant (p<0.03) for all three fitted curves and had a similar shape to the tertiary umbel level curve (Figure 3.2).

3.3.3. Seed yield

3.3.3.1. Seed yield per area (grams m^{-2}) for all umbel levels combined

Seed yield (g m^{-2}) data were $\log_e(x+1)$ transformed. There was a significant statistical difference (p<0.001) and huge biological difference between cultivars with A yielding 18 and B 192 g m^{-2} (transformed means A 2.7, B 5.2 g m^{-2}, $LSD_{0.05}$ 0.5). Both density (p=0.016) and the interaction (p=0.020) also had significant effect on yields (g m^{-2}) with cv. A unaffected by density, while seed yield of B showed a considerable increase (Table 3.3 and Figure 3.3). As only two samples of cleaned seed were collected from quaternary level umbels, they were thus excluded from all further analysis.

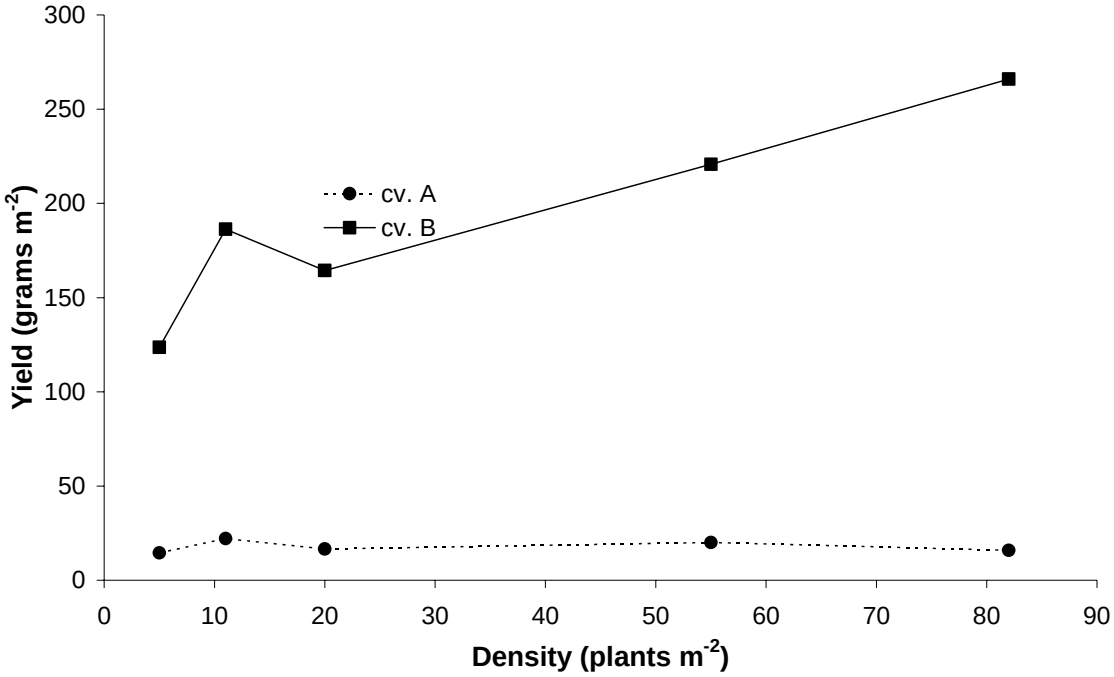

Figure 3.3. Effect of plant density (plants m^{-2}) on the yield (g m^{-2}) of two carrot F1 seed parent cultivars (A and B).

There was a significant difference (p=0.001) between the linear trend of the cultivars with the yield of cv. A static regardless of density, while B had a strong linear increase in yield with increasing density (Figure 3.3).

Table 3.3. Effect of plant density (plants m^{-2}) on $Log_e(x+1)$ transformed seed yield (g m^{-2}) for all umbels for two carrot F1 seed parent cultivars (A and B).

	Density (plants m^{-2})						
	5	**11**	**20**	**55**	**82**	**C-$LSD_{0.05}$**	**D-$LSD_{0.05}$**
A	2.6	3.0	2.6	2.9	2.5	0.70	0.61
B	4.8	5.2	5.1	5.3	5.5		

3.3.3.2. Seed yield per plant (grams $plant^{-1}$) for all umbel levels combined

Seed yield (g $plant^{-1}$) data were transformed using $log_e(x+1)$. There was a significant difference between cultivars with cv. A yielding an overall mean of 0.4 and B 4.2 g $plant^{-1}$ (transformed means A 0.28, B 1.31, $LSD_{0.05}$ 0.227). Density also significantly affected seed yield ($p<0.001$) unlike the density × cultivar interaction ($p=0.074$). Seed yield of cv. B showed a considerable decline as density increased in contrast to cv. A (Table 3.4 and Figure 3.4).

Table 3.4. Effect of plant density (plants m^{-2}) on $Log_e(x+1)$ transformed seed yield (g $plant^{-1}$) for all umbel levels for two carrot F1 seed parent cultivars (A and B).

	Density (plants m^{-2})						
	5	**11**	**20**	**55**	**82**	**C-$LSD_{0.05}$**	**D-$LSD_{0.05}$**
A	0.6	0.4	0.2	0.1	0.1	0.22	0.27
B	1.8	1.6	1.3	1.0	0.9		

There was a significant difference ($p=0.007$) between the cultivars for the linear fitted curve with both experiencing an initial decline in yield with increasing density, which then flattened out at approximately 20 plants m^{-2} (Figure 3.4).

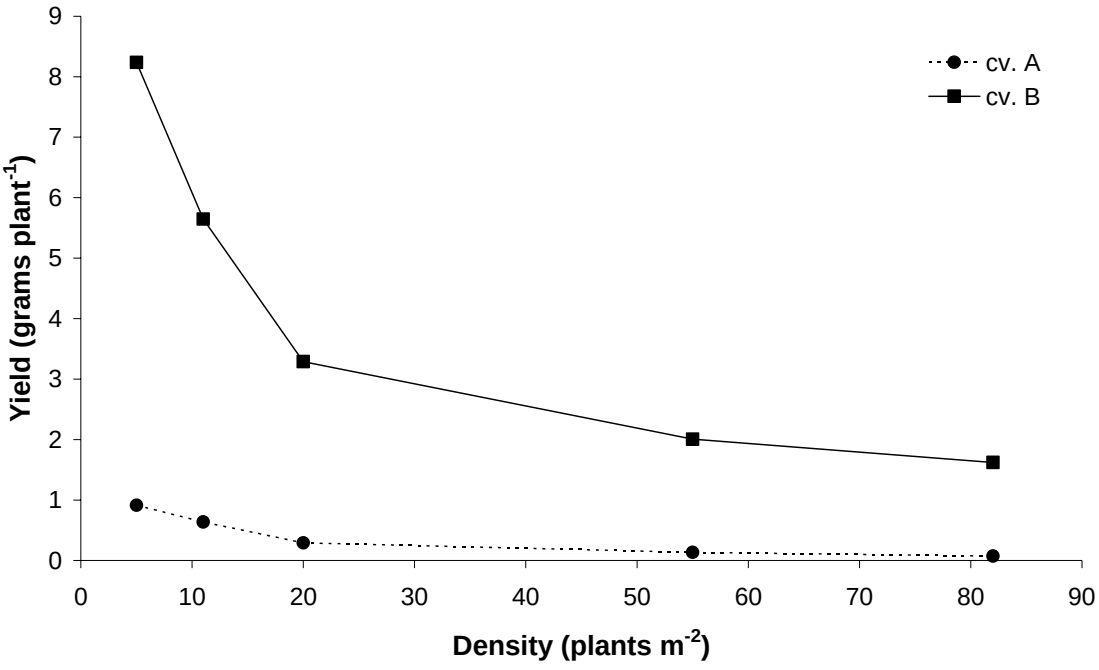

Figure 3.4. Effect of plant density (plants m^{-2}) on the yield (g $plant^{-1}$) of two carrot F1 seed parent cultivars (A and B).

3.3.3.3. Seed yield per area (grams m^{-2}) of each umbel level

Seed yield (g m^{-2}) data were $\log_e(x+1)$ transformed. For the primary umbel, there was a significant difference ($p<0.001$) between cultivars and among densities with cv. B yielding twice that of A, and with yield increasing ten fold from the lowest to highest densities (Tables 3.5, 3.6 and Figure 3.5). However, the yield × cultivar interaction was not significant ($p=0.091$). The yield for the secondary umbel level was also significantly different between cultivars ($p<0.001$) and among densities ($p=0.024$). However, in contrast with the primary umbel level, yield decreased with increasing density, but the decrease was only significant for cv. A. The density × cultivar interaction did not significantly affect yields ($p=0.629$) (Tables 3.5, 3.6 and Figure 3.5). The yield from tertiary level umbels was not significantly different between the two cultivars ($p=0.123$) but among the density levels ($p<0.001$) and for the density × cultivar interaction ($p<0.001$) with yield decreasing with increasing density. However, most of the decrease was due to cultivar B decreasing from 12 g m^{-2} to zero at a density of 55 plants m^{-2} (Tables 3.5, 3.6 and Figure 3.5).

Table 3.5. Seed yield (g m^{-2}) of each umbel level of two carrot F1 seed parents cultivars (A and B); values are means of five density levels.

		Untransformed	$\log_e(x+1)$ transformed	Transformed data $LSD_{0.05}$
U1	**A**	6	1.5	0.31
	B	66	3.9	
U2	**A**	10	2.2	0.38
	B	118	4.6	
U3	**A**	2	0.9	0.35
	B	6	1.1	

Table 3.6. Effect of plant density (plants m^{-2}) on the seed yield (g m^{-2}) of each umbel level of two carrot F1 seed parent cultivars (A and B).

		Untransformed data Density (plants m^{-2})					$\log_e(x+1)$ transformed Density (plants m^{-2})					C-LSD 0.05	D-LSD 0.05
		5	11	20	55	82	5	11	20	55	82		
U1	**A**	1	3	5	9	10	0.78	1.36	1.58	1.93	2.00	0.602	0.684
	B	15	27	38	104	155	2.73	3.32	3.59	4.60	5.03		
U2	**A**	10	15	11	8	5	2.37	2.59	2.28	2.07	1.45	1.738	1.847
	B	97	143	125	116	110	4.54	4.9	4.80	4.45	4.48		
U3	**A**	3	4	1	3	1	1.26	1.28	0.42	0.83	0.56	1.843	0.783
	B	12	16	2	0	0	2.31	2.67	0.73	0.0	0.0		

For the linear fitted curves, there was a significant difference ($p=0.006$) between cultivars for the primary umbel, but not for the quadratic or cubic curves ($p>0.7$). For cultivar B, the relationship was almost completely linear, while for A it was best explained by a quadratic equation (Figure 3.5). There was no difference between the

curves for A and B (p>0.1) for the secondary umbel level for any of the fitted curves, while the mean density curve had a significant linear trend (p=0.003). The secondary umbel level of both cultivars also displayed a pronounced peak at 11 plants m^{-2}, which was also visible for the tertiary level umbels although to a lesser extent . This effect was also present for plant height (Figure 3.1), although the peak for cv. A was not as pronounced. For the tertiary umbel level, there was a significant difference (p<0.001) between the cultivars for the linear fitted curve with the curve for cv. A undulating in a complex shape, while B reaches zero at a density level of 55 plants m^{-2} (Figure 3.5).

3.3.3.4. Seed yield per plant (grams plant^{-1}) of each umbel level

Seed yield (g plant^{-1}) data were $\log_e(x+1)$ transformed. For the primary umbel, there was a significant difference in yield between the two cultivars (p<0.001) and among the density levels (p=0.003), but not as a result of the interaction of the two (p=0.187). Overall, the seed yield of cultivar B was considerably higher than that of A, but only with a small biological decrease in yield with increasing density (Tables 3.7, 3.8 and Figure 3.6). For the secondary umbel, there was a significant difference as a result of cultivar, density and the interaction (p<0.001) (Tables 3.7, 3.8 and Figure 3.6). There was a huge biological difference in yield, particularly at the lower densities, with yield rapidly decreasing with increasing density, for both cultivars. For the tertiary umbels, there was a significant difference for cultivar (p=0.002), density (p<0.001) and their interaction (p<0.001), with a similar density response as the primary umbel except that yield was zero at higher densities (Tables 3.7, 3.8 and Figure 3.6).

Table 3.7. Seed yield (g plant^{-1}) of two carrot F1 seed parent cultivars (A and B); values are means of five density levels.

		Untransformed	$\log_e(x+1)$ transformed	Transformed data $LSD_{0.05}$
U1	**A**	0.21	0.184	0.1847
	B	2.23	1.142	
U2	**A**	0.84	0.499	0.2587
	B	8.41	1.859	
U3	**A**	0.20	0.162	0.1032
	B	0.79	0.405	

There was no significant difference (p>0.1) between the shape of the density curves for the primary umbel. There was a significant fit for the mean density curve to the linear fitted curve (p=000.1) with yield decreasing only slightly with increasing density (Figure 3.6). At the secondary umbel level, there was a significant difference (p<0.001) between the cultivars for the linear fitted curve, but, they were not significantly different for the quadratic (p=0.251) and cubic (p=0.760) fitted curves. The untransformed data

show exponentially decreasing yield with increasing density levels (Figure 3.6). The tertiary umbel level was similar to the secondary, with a significant difference ($p<0.001$) between cultivars for the linear fitted curve, showing a rapid decline in yield from the lowest densities to zero at higher densities (Figure 3.6).

Table 3.8. Effect of plant density (plants m^{-2}) on the seed yield (g $plant^{-1}$) of each umbel level of two carrot F1 seed parent cultivars (A and B) at five densities.

		Untransformed data Density (plants m^{-2})					$Log_e(x+1)$ transformed Density (plants m^{-2})					C-LSD 0.05	D-LSD 0.05
		5	11	20	55	82	5	11	20	55	82		
U1	A	0.3	0.3	0.2	0.2	0.1	0.22	0.25	0.20	0.14	0.11	0.222	0.177
	B	3.0	2.5	1.9	1.9	1.9	1.36	1.23	1.03	1.04	1.05		
U2	A	2.1	1.4	0.6	0.2	0.1	1.09	0.80	0.42	0.14	0.06	0.401	0.385
	B	19.3	13.0	6.3	2.1	1.4	2.97	2.59	1.96	1.00	0.79		
U3	A	0.6	0.3	0.1	0.1	0.0	0.43	0.26	0.05	0.06	0.02	0.284	0.305
	B	2.4	1.5	0.1	0.0	0.0	1.10	0.85	0.08	0.00	0.00		

3.3.3.5. Proportional contribution (percent) of each umbel level to yield

The percentage contribution of each umbel level to yield was calculated by dividing the yield of each umbel level by the sum of yield for each density. Due to low sample numbers of tertiary level umbels, these were excluded from the calculations (Figure 3.7). Converting yield into proportional contribution equalises the scale of the two cultivars' responses, which permits a fairer comparison. However statistical analysis is not possible. Figure 3.7 is similar to Figure 3.5; however, calculating the proportional contribution of each umbel level resulted in elimination of the suspected sampling artefacts for 11 plants m^{-2} and showed that the two cultivars responded similarly to changing density levels, with the primary and secondary umbel levels showing comparable responses in terms of the curve shape and the starting and ending percentage contribution.

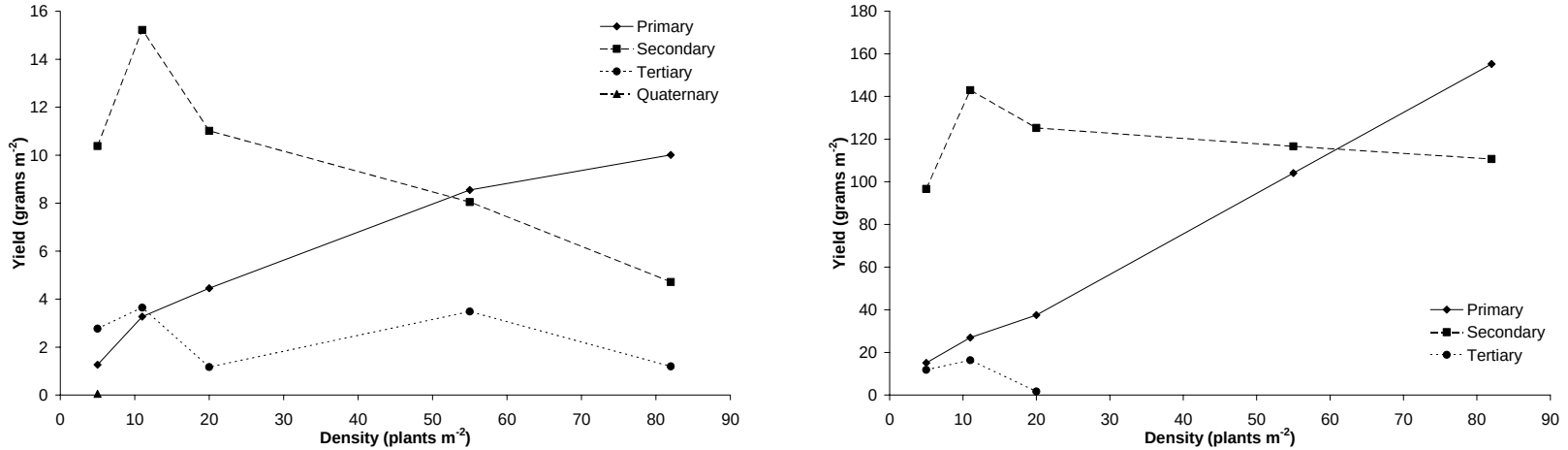

Figure 3.5. Effect of plant density (plants m^{-2}) on yield (g m^{-2}) for each umbel level of two carrot F1 seed parent cultivars (cv. A left, B right) (untransformed data). N.B. dissimilar X-axis scales.

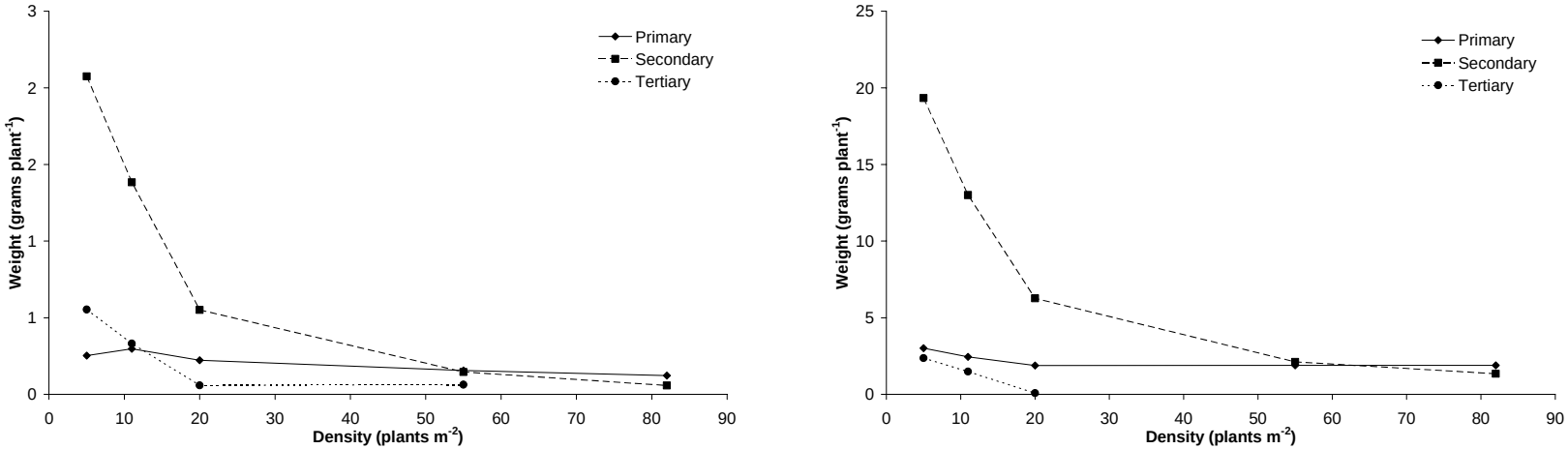

Figure 3.6. Effect of plant density (plants m^{-2}) on yield (g $plant^{-1}$) for each umbel level of two carrot F1 seed parent cultivars (cv. A left, B right) (untransformed data). N.B. dissimilar X-axis scales.

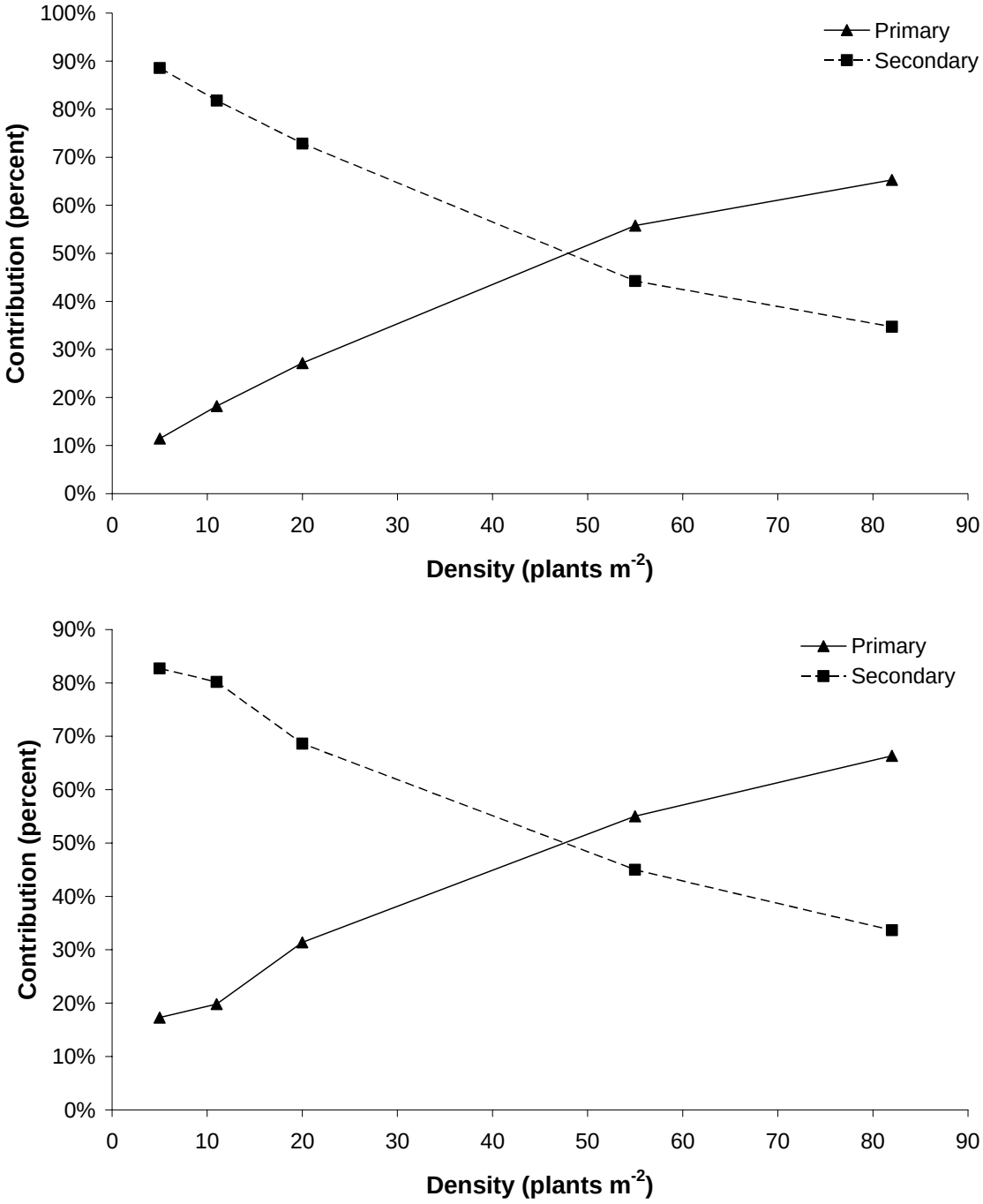

Figure 3.7. Effect of plant density (plants m^{-2}) on proportional contribution to yield (%) for the primary and secondary umbel levels of two carrot F1 seed parent cultivars (cv. A top, B bottom) (calculated data).

3.3.4. Germination

Data were normally distributed so untransformed data were used in the REML analysis. As REML analysis does not produce LSD values, standard errors of differences (SE) are given. For the cultivar × density interaction, there are separate SE for comparing between cultivars and among densities and they are presented as average, maximum and minimum SE. All Chi sq. p values given are approximations.

At the primary umbel level there was a significant difference between cultivars and for the cultivar × density interaction (Chi sq. $p<0.001$) with cv. A having a considerably lower germination than B, and, unlike B, showing decreasing germination with increasing density. There was no significant difference among the different densities (Chi sq. $p=0.606$) (Tables 3.9, 3.10 and Figure 3.8). At the secondary umbel level, there were no data for cv. A at 50 plants m^{-2}, therefore no predictions could be made for the main effects of cultivar and density. However, as observed for the primary umbel level, the interaction between the two effects was significant (Chi sq. $p<0.001$) with cv. B having an overall higher percentage germination than A. Cultivar B maintained a similar percentage germination regardless of density, while for A there was a considerable reduction in germination with increasing density (Tables 3.9, 3.10 and Figure 3.8). The analysis of the tertiary umbel level was restricted to the 5 to 20 plants m^{-2} densities as both cultivars had produced insufficient seed for testing at higher densities. There was a significant difference between cultivars (Chi sq. $p<0.001$) with cv. B having a higher percentage germination than A but there were no significant differences among densities (Chi sq. $p>0.4$) or as a result of the interaction (Chi sq. $p>0.8$) (Tables 3.9, 3.10 and Figure 3.8).

Table 3.9. Germination (%) of each umbel level of two carrot F1 seed parents (A and B); values are means of five density levels.

		Germination (%)	SE
U1	**A**	67	2.8
	B	93	
U2	**A**	n/a	n/a
	B	86	
U3	**A**	42	7.4
	B	78	

Table 3.10. Effect of plant density (plants m^{-2}) on the germination (%) of each umbel level of two carrot F1 seed parent cultivars (A and B) at five densities.

		Density (plants m^{-2})								
		5	**11**	**20**	**55**	**82**		**Ave. SE**	**Max. SE**	**Min. SE**
U1	**A**	77	73	69	58	56	**cv.**	4.0	6.5	2.9
	B	91	93	95	94	94	**Density**	4.6	6.2	3.7
U2	**A**	53	64	60	n/a	24	**cv.**	5.5	7.7	5.3
	B	94	89	84	83	80	**Density**	6.0	7.6	5.3
U3	**A**	40	37	47	n/a	n/a	**cv.**	12.3	17.1	8.7
	B	78	78	78	n/a	n/a	**Density**	12.1	17.6	8.7

For the primary umbel level, there was a significant difference (Chi sq. $p<0.001$) between the cultivars for the linear fitted curves, with cv. B being nearly linear, while A showed a quadratic trend: increasing at first, then decreasing before levelling off

(Figure 3.8). The secondary umbel level also showed a significant difference between cultivars for both the linear (Chi sq. p=0.001) and quadratic (Chi sq. p=0.005) fitted curves with cv. B maintaining a high level of germination regardless of density, while A declined considerably (Figure 3.8). At the tertiary umbel level, there was no significant difference between the cultivars for any curve (Chi sq. p>0.7), neither was there a significant fit of the mean density curve at the linear or quadratic level (Chi sq. p>0.7). The two curves appear different on visual assessment, so it is unclear why they did not differ statistically (Figure 3.8).

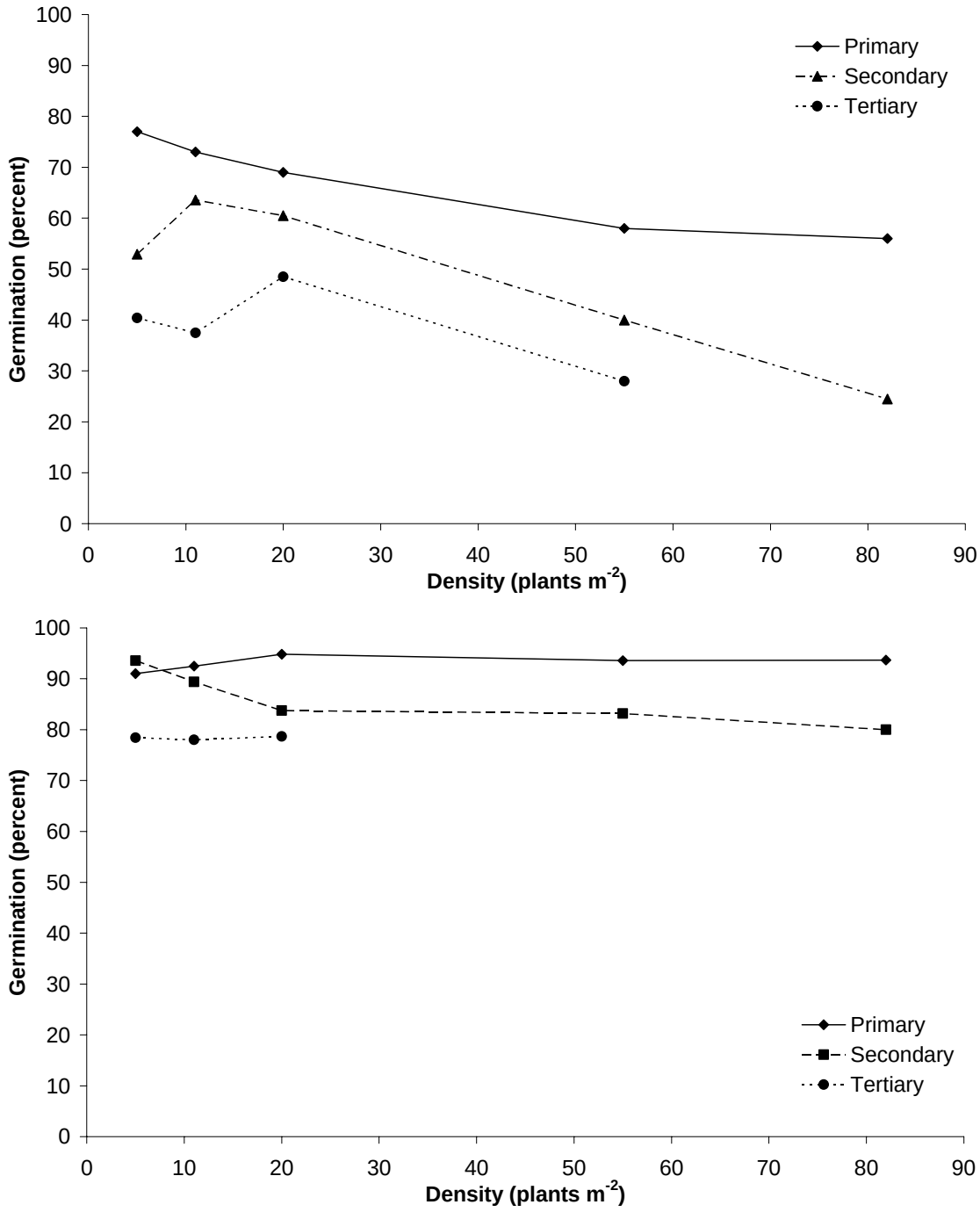

Figure 3.8. Effect of plant density (plants m^{-2}) on germination (%) for each umbel level of two carrot F1 seed parent cultivars (cv. A top, B bottom) (untransformed data).

Overall germination was calculated by firstly estimating the proportion of seed each umbel level would have contributed to a bulk seed lot, if the umbels had been harvested together. This proportion is based on the number rather than the weight of seeds, and was calculated from the thousand seed weight (TSW) (for a detailed discussion of the rationale for this see section 3.5). The proportion of contribution of each umbel level to the bulk seed lot was multiplied by the percentage germination, and the values for all umbel levels were added up to provide the overall germination (Figure 3.9). No statistical comparison of the data is possible and the results should be treated as indicative only. The results for the two cultivars were quite different, with the overall germination of cv. B unaffected by plant density, while A showed the highest germination rate at a density of around 15 plants m^{-2} followed by a decrease at higher densities.

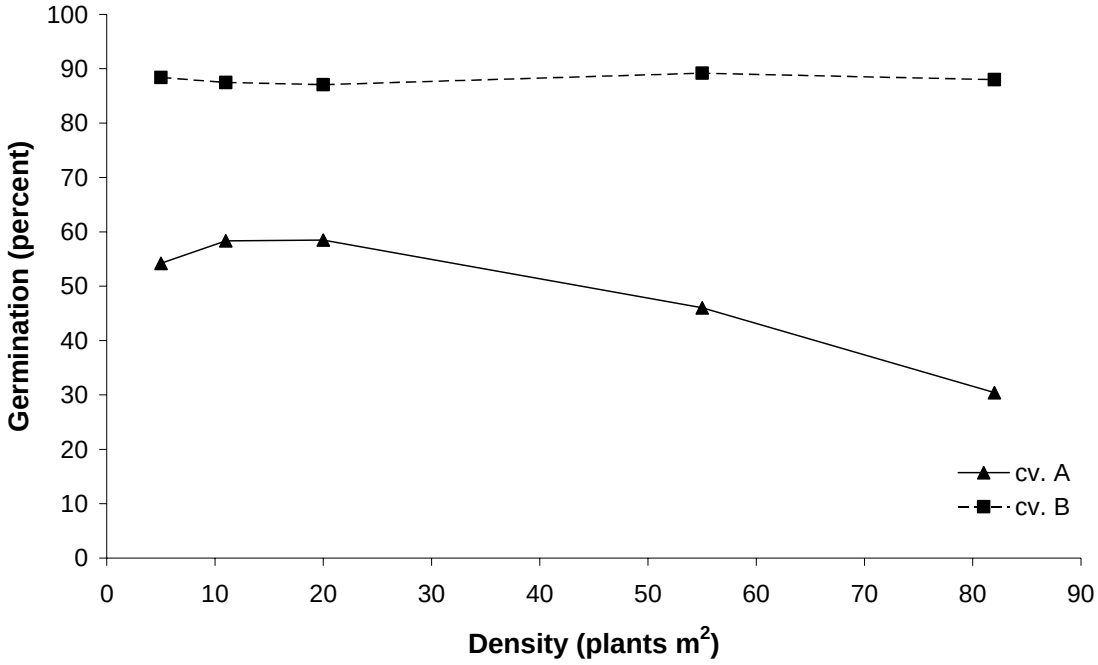

Figure 3.9. Effect of plant density (plants m^{-2}) on germination (%) for all umbel levels of two carrot F1 seed parent cultivars (A and B) (calculated data).

3.3.5. Seed vigour

The seed from cv. A had poor germination indicating low vigour. It was therefore not tested, and no comparison between cultivars could be made. The analysis of the effect of plant density on seed vigour of cv. B was only completed for the primary and secondary umbel levels, as there was insufficient data to analyse the tertiary umbel level. The tertiary umbel data were, however, included in Figure 3.10. Data were normally distributed and the arcsine transformation did not alter the results, so only untransformed data are presented.

There was a significant effect for umbel level (p=0.011) with the primary umbel level having a vigour of 87% and secondary umbel level of 82% ($LSD_{0.05}$ 4.3). Neither density nor the density × umbel interaction were significant, even though the change in seed vigour was greater than between umbel levels (Table 3.11 and Figure 3.10). The density curve had a significant fit (p=0.034) for the quadratic curve with it initially increasing with increasing density up to 55 plants m^{-2} followed by a decrease. A calculated, combined umbel vigour, based on TSW, shows vigour initially rising with increasing density then decreasing at 82 plants m^{-2} (Table 3.11).

Table 3.11. Effect of plant density (plants m^{-2}) on vigour score (%) of carrot, F1 seed parent cultivar B, for both umbels combined (calculated), and the density × umbel level interaction.

	Density (plants m^{-2})						
	5	**11**	**20**	**55**	**82**	**$LSD_{0.05}$**	**p value**
Calculated combined umbel levels	84	82	85	89	83	n/a	n/a
U1	86	81	87	93	90	9.4	0.085
U2	80	84	85	85	74		

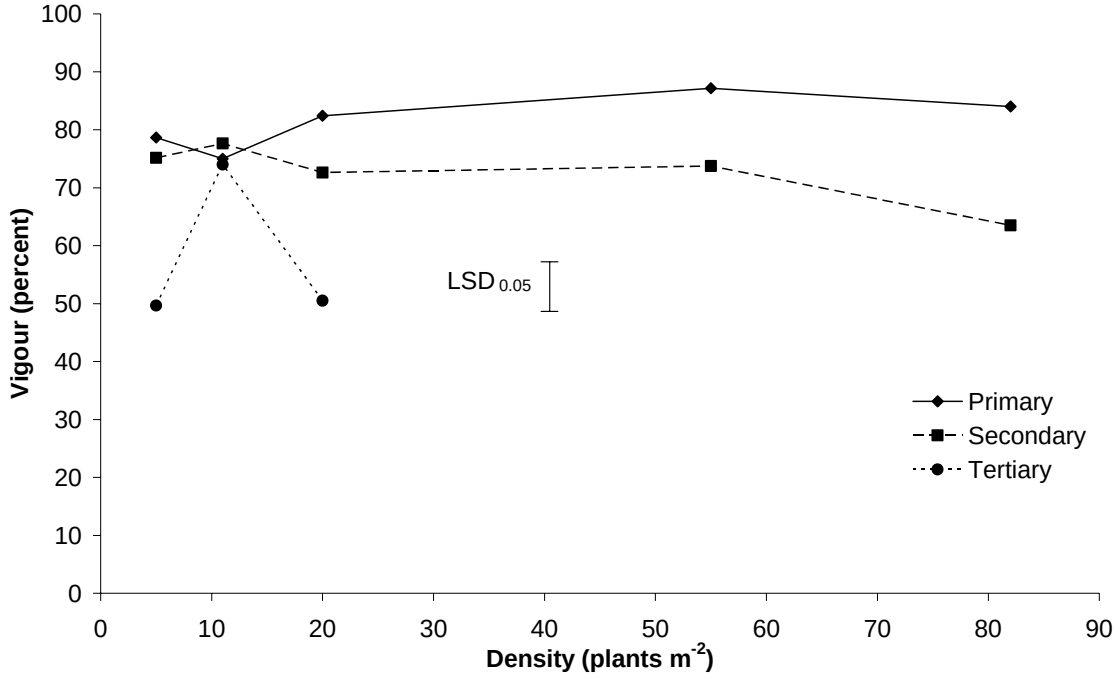

Figure 3.10. Effect of plant density (plants m^{-2}) on vigour score (%) for three umbel levels of one carrot F1 seed parent: cultivar B (untransformed data).

3.3.6. Thousand seed weight (grams)

Data for the primary umbel level were $\log_e(x)$ transformed before analysis by REML. The secondary and third order umbels did not require transformation. Both the transformed and exponential, back-transformed results for the primary umbel levels are presented. For the second and third order umbels, only untransformed data are presented.

Cultivar (Chi sq. p=0.029), density (Chi sq. p=0.007) and the interaction of the two factors (Chi sq. p=0.001) had a significant effect on TSW of the primary umbel level. However, the difference was not biologically significant with only 0.1 g difference between cultivars. The effect of density on the seed weight of the two cultivars was also ambiguous, with cv. B initially rising to a maximum at 20 plants m^{-2} and falling back while A oscillated (Tables 3.12, 3.13 and Figure 3.11). No cultivar comparison could be made for the secondary umbel level due to cv. A having no results at 80 plants m^{-2}, while the cultivar × density interaction was significant for this umbel level (Chi sq. p=0.002). Nevertheless, the biological effects were not as clear, and closely mirrored the results for the primary umbel level, with the TSW of cv. B initially increasing, this time to 11 plants m^{-2}, and then decreasing, while the TSW of A dropped to a low between 20 and 55 plants m^{-2} (Tables 3.12, 3.13 and Figure 3.11). For the tertiary umbel level, analysis was restricted to densities of 5, 11 and 20 plants m^{-2}, and only the cultivar effect differed significantly (Chi sq. p<0.001). However, as observed for the other two umbel levels, the difference of 0.2 g was not biologically large (Tables 3.12, 3.13 and Figure 3.11).

Table 3.12. Thousand seed weight (grams) of each umbel level of two carrot F1 seed parent cultivars (A and B) averaged over five densities. For U1, log_e(TSW)-transformed data and the exponential back transformation are presented. U2 and U3 values are untransformed.

		TSW (grams)	Log_e(TSW)	SE
U1	**A**	1.2	0.19	0.04
	B	1.3	0.29	
U2	**A**	n/a	n/a	n/a
	B	1.0	n/a	
U3	**A**	1.2	n/a	0.08
	B	1.0	n/a	

Table 3.13. Effect of plant density (plants m^{-2}) on thousand seed weight (grams) of each umbel level of two carrot F1 seed parent cultivars (A and B) at five densities. Log_e(U1) is transformed data, U1 is an exponential back transformation of the Log_e(U1) results. U2 and U3 are untransformed.

		TSW (grams)								
		5	**11**	**20**	**55**	**82**		**Ave. SE**	**Max. SE**	**Min. SE**
Log_e	**A**	0.30	0.25	0.01	0.26	0.10	**cv.**	0.085	0.138	0.064
(U1)	**B**	0.29	0.36	0.40	0.22	0.17	**Density**	0.863	0.124	0.068
U1	**A**	1.3	1.3	1.0	1.3	1.1	**cv.**	n/a	n/a	n/a
	B	1.3	1.4	1.5	1.2	1.2	**Density**	n/a	n/a	n/a
U2	**A**	1.3	1.3	1.1	n/a	1.4	**cv.**	0.07	0.10	0.07
	B	1.0	1.1	1.0	0.9	0.8	**Density**	0.08	0.10	0.07
U3	**A**	1.2	1.2	1.3	n/a	n/a	**cv.**	0.13	0.19	0.1
	B	0.9	1.0	1.0	n/a	n/a	**Density**	0.13	0.19	0.10

For the curve fitting, there was no significant difference between the cultivars' primary umbel levels for the linear (Chi sq. p=0.648) and quadratic (Chi sq. p=0.168) fitted

curves. However, the cubic fitted curve was significantly different between cultivars (Chi sq. p<0.001), which is reflected in the oscillating nature of the curves, particularly for cv. A (Figure 3.11). For the secondary umbel level, there were significant differences between cultivars for both linear (Chi sq. p=0.003) and quadratic (Chi sq. p=0.020) fitted curves reflecting the large difference in the two curves' shapes (Figure 3.11). No curve fitting was possible for the tertiary umbel level.

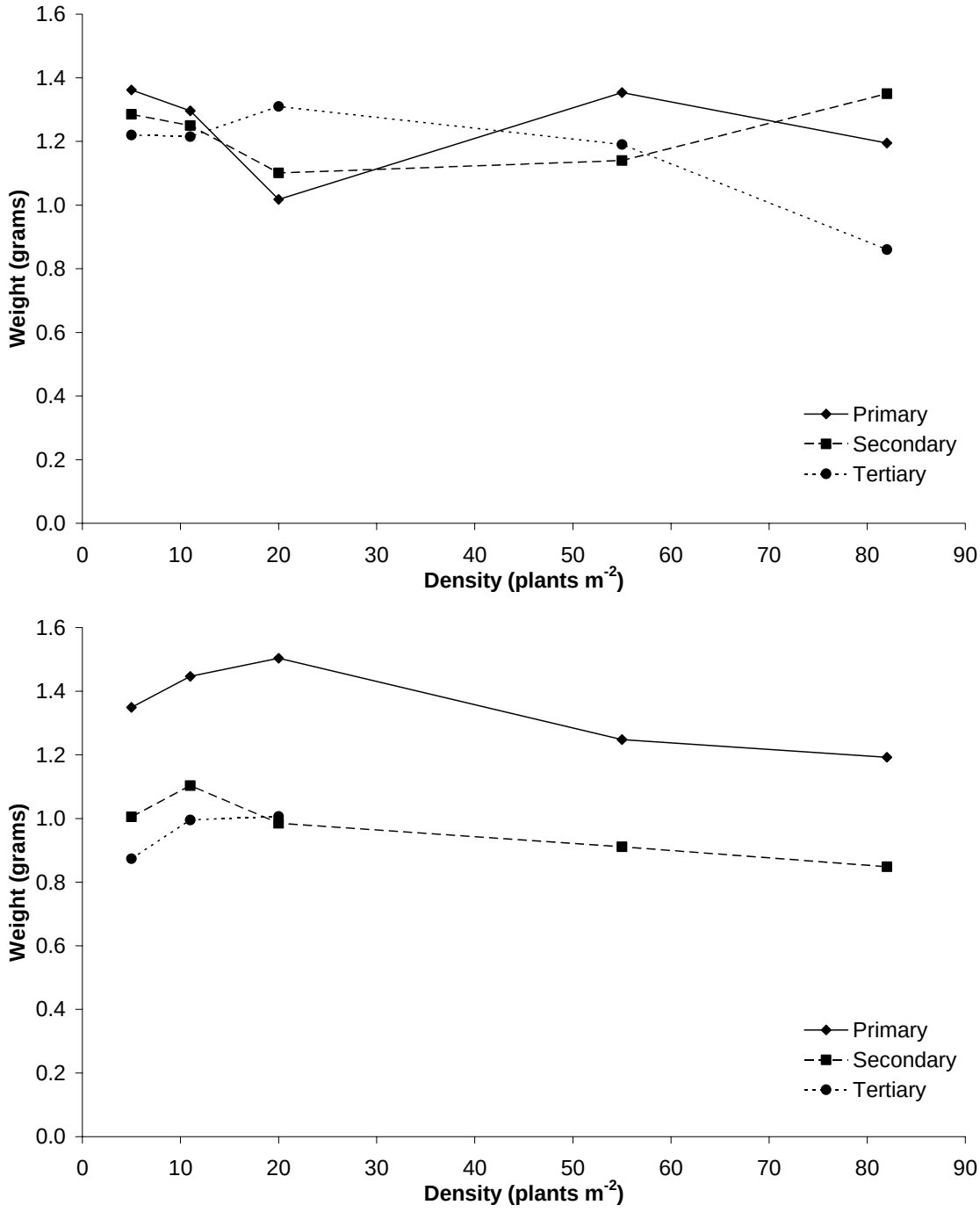

Figure 3.11. Effect of plant density (plants m^{-2}) on thousand seed weight (grams) of two carrot F1 seed parent cultivars (cv. A top, B bottom) (untransformed data).

3.3.7. *A. radicina* and *A. dauci* seed infestation

Data were normally distributed and arcsine transformation did not alter the results, so only untransformed data are presented. There was a significant difference ($p=0.005$) in the percentage of *A. radicina* infested seeds among the different densities and there was a significant fit ($p<0.001$) for the linear curve with the percentage increasing with increasing density (Table 3.14). No seed was found with *A. dauci* infestation.

Table 3.14. The effect of plant density (plants m^{-2}) on percent carrot seed infested with *A. radicina*.

	5	11	20	55	82	$LSD_{0.05}$
Infested seed (%)	6.1	5.6	7.3	8.1	12.0	3.28

3.4. Discussion

The two cultivars clearly vary in some of their responses to increasing density, which was one of the key hypotheses of this research. However, the very large difference in seed yield indicates that there may have been sub-optimal pollination. This means that care should be exercised when making comparisons between the cultivars where any difference between them could be due to the large yield difference. At the same time, the cultivars also showed considerable similarities, despite the large yield differences.

3.4.1. Plant height

Both cultivars showed an initial slow height increase at densities between 5 and 10 plants m^{-2} followed by a rapid increase between 10 and 20 plants m^{-2} and a slower linear increase at higher densities. A plant grown without any competition would be expected to grow to a genetically predetermined height and there will be a range of densities where inter-plant competition is minimal, i.e. has no effect on height. As densities increase competition starts, resulting in increasing height, until the plants reach their genetic maximum, which usually results in a sigmoidal relationship between height and density. In this trial, the carrot plants only just covered the ground area available at the lowest density, but at a density of 10 plants m^{-2} they had grown into each other. This indicates that Figure 3.1 is a sigmoidal height response to increasing density and that both cultivars show a similar response in terms of the inflection points of the curves but at differing heights. These results are similar to those of Oliva *et al.* (1988), who found that for spacings of 4 to 35 plants m^{-2} height increased from 1.3 to 1.6 m with a plateau at a density of 17 plants m^{-2}.

3.4.2. Umbel numbers

While the cultivars clearly differed in height, the assumption that they would differ in the number of umbels was clearly not the case, except for the quaternary umbel level. It

has to be considered that the contrasting heights may have influenced the anecdotal impression the seed supplier had of the umbel numbers.

The density curves for umbel numbers (Figure 3.2) also followed a similar pattern to the height response (Figure 3.1) in that there was a large reduction in umbel number between plant densities of 5 and 20 plants m^{-2}. This is a further indication that plant competition starts at or under 5 plants m^{-2} with a rapid increase of competitive effects which start to diminish above densities of 20 plants m^{-2}. The similarity of the two cultivars' response in terms of umbel numbers to increasing density, both statistically and biologically, is very clear despite the observed differences in height.

3.4.3. Seed yield

While seed yield showed some substantial differences between the cultivars, there were also some interesting similarities. The comparison of the total yield for all umbels showed a large biological difference. The extremely low yield of cv. A suggests that this difference may be due to more than just a cultivar effect and that other factors may influence the result. The fact that the two cultivars were grown in close proximity in the radial trial, suggests that environmental factors are unlikely to be responsible. However, ensuring that the pollen parent is producing pollen at the same time that the seed parent is able to receive the pollen can be quite difficult. Carrot seed growers and their industry advisors put considerable effort into trying to achieve this task including trimming umbels from some or all of the pollen parent plants (James Smith, pers. comm. 2003). It is possible that a miss match between the flowering times of the pollen parent and cv. A is the reason why its seed yield was low.

The yield per $meter^2$ (Figure 3.3) for all umbels combined shows the greatest difference between the cultivars, with cv. A showing almost no response, while B doubled with increasing density. This flat response of cv. A may well have been affected by the overall low yield. However, the lack of increase in yield with increasing density was unexpected. The combined umbel yield per plant (Figure 3.4) showed a similar trend for the two cultivars with an initial decline in yield, which then levelled off at a density of 20 plants m^{-2} with cv. A decreasing by 89% and B 80%. Despite this the curves are statistically different for the linear fit.

Both these results and those for umbel numbers and height indicate that 20 plants m^{-2} is a threshold, where inter-plant competition changes from rapidly increasing competitive effects to a situation, where increasing competition results in more gradual or no change. However, while plant height showed a sigmoidal response, indicating the onset

of competition occurs at 10 plants m^{-2}, both umbel numbers and yield already revealed a strong decline at 5 plants m^{-2}, indicating that inter-plant competition for these factors started at even lower densities. This would indicate that umbel numbers and yield are more sensitive indicators of competition than plant height. These results also indicate that densities beyond 20 plants m^{-2} may produce diminishing marginal returns, e.g., each extra unit of seed planted results in a smaller increase in yield.

The seed yield (both per plant and per $meter^2$) of each umbel level showed a considerable density effect with both cultivars displaying broadly similar responses despite the large differences in yield. For yield per $meter^2$, as density increased the proportion of the seed from the primary and secondary umbel levels increased considerably. For yield per plant the contribution of the primary umbel decreased only marginally, while the secondary order umbels' yield decreased sharply. This clearly shows that seed production by the primary umbel level was mostly unaffected by density, while the tertiary umbel level was affected to a greater extent. It was, however, the secondary umbel level that was predominately responsible for the changing distribution of seed among the umbel levels.

For the yield per $meter^2$ of individual umbel levels (Figure 3.5), there was a statistical difference between the primary umbel curves of the two cultivars with B showing a strong linear response, compared to the more quadratic response of cv. A, that showed flattening off as density increased. However, the secondary umbel level curves were statistically the same. For yield per plant (Figure 3.6), the reverse happened with no statistical difference between the primary umbel level curves but a significant difference at the secondary umbel level. These changes in the significance of effects on the primary compared to the secondary umbel level depending on the unit of yield used is evidence that the differences between cultivars are quite subtle.

The tertiary umbel level showed the largest difference with significant differences between the curves for both yield measurements and with cv. A producing seed even at 82 plants m^{-2}, while for B yield production had ceased at 55 plants m^{-2} (Table 3.8). This result indicates that the industry perception that cv. A produces more lower order umbels, may be based on umbel size at the tertiary umbel level rather than on the actual number of umbels, assuming, that as there is more seed produced at the tertiary umbel level by cv. A its umbels must be larger. No measurement of the size of umbels was undertaken, but if there is a visible size difference between cultivars for lower order

umbels, particularly at the tertiary umbel level, this may well give the visual impression of more lower order umbels as the smaller umbels will not be easily visible.

The similarities between the two cultivars is at its clearest for the calculated comparison of proportion of yield per umbel level with the exclusion of the tertiary umbel level (Figure 3.7). The two cultivars responded in a very similar way, despite the very large difference in yield. This indicates the two cultivars had a similar underlying response to increasing density.

These results are helpful in interpreting the results of previous research, which sometimes appears to present conflicting results. For example, there was little effect on yield (g m^{-2}) between densities of 3 and 11 plants m^{-2} (Sharma & Singh, 1981), which is not unexpected as this trial indicates that inter-plant competition is only starting at these densities. In contrast, yield (g m^{-2}) was found to increase nearly linearly from 110 to 150 g m^{-2} from densities of 10 to 80 plants m^{-2} (Gray, 1981), which is similar to that observed for cv. B in this research, except that the maximum yield was 266 g m^{-2}. In another study, contribution to yield from the primary umbel increased from 20 to 60% with increasing density from 4 to 36 plants m^{-2} (Oliva *et al.*, 1988), while in this study, for cv. A it increased from 7 to 45% from 5 to 55 plants m^{-2} and for B from 12 to 47% at the same densities. In contrast, 12 plants m^{-2} gave the highest yield in trials in California from a density range of 2 to 36 plants m^{-2}, with yield plateauing at 30 plants m^2 (Oliva *et al.*, 1988), which is at odds with the results presented here. Clearly, comparing studies with densities below 10 plants m^{-2} with those at higher densities is problematic as inter-plant competition differs considerably with varying densities. However, there are still a number of studies that present contradictory results e.g., (Gray, 1981) and (Oliva *et al.*, 1988) (above), and studies reporting multiple experiments that find yield increases in some but not others (Noland *et al.*, 1988). Studying the two cultivars in this trial also produced conflicting results for gross yield, both in total value and response to density, indicating that there may well be considerable variation among cultivars, although the very low yield recorded for cv. A means the results are not conclusive. Nonetheless, the proportion of yield (Figure 3.7) that the primary and secondary umbels contributed across all five densities was very similar for both cultivars . This is an indication that some crop responses to density (e.g., yield) may be affected by a range of other environmental factors, while others (e.g., distribution of seed between primary and secondary umbels) are more directly determined by density, while environmental factors have limited impact.

3.4.3.1. Data artefacts

The peak observed in yield per plant for both cultivars at 11 plants m^{-2} (Figures 3.3 and 3.5) was not apparent in the yield per $meter^2$ (Figure 3.4) although both graphs are based on the same data. Other results also showed similar results, that could be viewed as 'anomalies' in the curves' shape , i.e., a deviation from the trend in the rest of the curve, around the 10 plants m^{-2} density, for example vigour (Figure 3.10) and germination of cv. A. (Figure 3.8). There is no obvious biological reason for this effect, and it is suggested that it is a sampling artefact. However, if future carrot seed density trials cover a similar range of densities, it may be valuable to extend the lowest density below 5 plants m^{-2} to determine at what density the competition effects become first noticeable and to provide more data points at lower densities in order to see if the results obtained from this study are anomalous, or if there is a real biological effect.

3.4.4. Germination

There was a considerable difference in germination between cultivars both in level and the density response. While a mismatch in the timing of the pollen and seed parents would be expected to produce lower yield, this is not true for germination. An argument could be made for the reverse; a poor seed set could mean that available plant resources are divided among fewer seeds, and that the remaining ones receive more resources, which could result in higher germination. The reason for the lower germination of cv. A is therefore unclear, and as such, it is considered unsuitable to base conclusions regarding seed quality on it. A key argument for using higher plant densities is that it will increase seed quality of bulk harvested seed, because the proportion of seed produced on the primary umbel level will increase and primary umbels produce higher quality seed (Jacobsohn & Globerson, 1980; Gray, 1981). However, because the umbels levels were harvested and analysed separately it is difficult to determine the properties (e.g., germination, vigour etc.) of the bulk seed lot they would of formed had the umbel levels been harvested together. This is discussed further in section 3.5.1. The results for the individual umbel levels for cv. B show that there has been an increase in germination from 91% to 94% with increasing density for the primary umbel while the opposite has occurred for the secondary umbel level with germination decreasing from 94% to 80%. At the same time the proportion of seed from the two umbel levels has altered considerably from 13% and 87% for the primary and secondary umbel levels respectively to 58% and 42% respectively (Figure 3.7). This change in proportionality is in keeping with the hypothesis of Grey (1981) and Jacobsohn & Globerson (1980) and it appears that this, in combination with the higher germination of the primary

umbel at higher densities may result in improved germination had the seed been harvested in bulk.

3.4.5. Vigour of cultivar B

While there was a significant difference between the primary and secondary umbel levels of five percentage points, the biological change was only moderate. The lack of statistical difference for density or the density × cultivar interaction, and the lack of a clear trend in the data, particularly at the lowest densities, where there appears to be a sampling artefact at 10 plants m^{-2}, indicates that density did not cause a large change in vigour. This is backed up by the calculated vigour for the combined umbel levels (Table 3.11) where there was an initial rise in vigour to 55 plants m^{-2} but then a drop at 82 plants m^{-2}. The tertiary umbel level, which was not included in the analysis due to insufficient samples, showed no clear biological trend, instead it may be a result of the 10 plants m^{-2} sampling artefact as discussed previously (section 3.4.3.1). The results up to 55 plants m^{-2} are in agreement with the hypothesis that increasing density will increase seed quality, but not the highest density.

3.4.6. Thousand seed weight

The typical TSW range of commercial carrot lots is 0.8 to 1.7 g (Bleasdale & Salter, 1982), which means that the TSW observed for the two cultivars falls around the mean of those values. Although they were statistically different, the lack of biologically significant differences between cultivars at the primary umbel level is compounded by the lack of clear biological trends in cv. A where the values for all three umbels oscillated considerably, which may be due to the limited number of samples. Cultivar B, in comparison, did show clear trends, with the primary umbel level peaking at 20 plants m^{-2} and the secondaries at 10 plants m^{-2}. There was a general decrease of TSW at the higher densities, which is confirmed by the statistical difference for the umbel × density interactions of both primary and secondary umbel levels. These data do not support the argument that increasing density increases seed quality.

3.4.7. *A. radicina* and *A. dauci* seed infestation

Increasing crop density resulted in a near doubling of the percentage of infested seed. While this result is from only the secondary umbel level of cv. B and should not be considered representative of the other cultivar or umbel levels, it shows an additional factor that can result in a decrease of seed quality as density increases. Further, it is considered notable that this effect was so pronounced over the small physical distance of this trial as it was expected that conidia would be moving freely throughout the crop

thereby creating similar infestation levels among all densities. However, the difference in plant growth between the lowest and highest densities was considerable, as reflected in the height and number of umbel data, but also in the field, where the lower density plants had an open branched structure, while at high densities the carrots were thin and stick-like with a dense mass of foliage. This suggests that if the levels of airborne conidia were distributed reasonably evenly over the trial area, the thicker foliage of the higher densities may have increased humidity and leaf wetness in the crop canopy (as discussed in the literature review section 2.2.2). This in turn may have increased the length and number of infestation periods, which could have lead to greater seed infestation levels at higher densities. This aspect would benefit from a more detailed study, especially recording the crop microclimate and correlating the data with infestation levels.

3.5. Conclusions

The hypothesis of Jacobsohn & Globerson (1980) and Gray (1981) that, in order to improve the seed quality of carrots, plant density should be increased, is supported by these results. The main part of their argument is:

- larger seed is of higher quality than smaller seed, and
- the primary umbel produces the largest seeds, and
- seed from the primary umbel is of higher quality than seed of the same size from lower order umbels, and
- increasing density increases the proportion of seed produced by the primary umbel:
- therefore, increasing density will result in higher seed quality when seeds from all umbels are mixed.

These results unambiguously support the majority of these points. The primary umbels had larger seed than lower order umbels and that seed had higher germination and vigour than lower order umbels. Increasing density dramatically increased the proportion of seed produced by the primary umbel. For cv. B there was also a clear increase in yield with increasing crop density. It also appears that germination increased with increasing density, however, due to the separation of umbel levels this is an inferred result.

The hypothesis does however includes the unstated assumption that the only effect of increasing density will be on the distribution of seed among the umbel levels, while it will not have any effects on the carrot plants as such or the amount of resources

(nutrients, energy) devoted to seed production. Stated explicitly, this is quite an assumption to make for a biological system, and one which should be verified experimentally .

3.5.1. The contribution of individual umbel levels to the properties of combined umbel seed lots

However, no trials have been found in the peer reviewed, primary literature, that have explicitly set out to measure the effect of carrot crop density on seed quality of a bulk seed lot (comprising mixed seed from all umbel levels) as produced by a commercial harvester. This is problematic as the process of cleaning, processing and measuring the different umbel orders separately means that the properties of the bulk seed lot cannot be easily, or at least accurately, be recreated. This is because the contribution that each umbel order makes to the properties of the bulk lot are in proportion to its percentage contribution to the bulk lot. However, the choice of measurement unit used to determine each umbel level's contribution to the bulk seed lot is critical. Weight (yield) of seed is easy and quick to measure. However, it is extremely unlikely that different umbel levels will have exactly the same average seed weight, so equal weights of seed from different umbel levels are almost certain to contain a different number of seeds. As all seed quality tests are completed on a specific number of seeds rather than a set weight of seeds, weight is, therefore, an inappropriate measure. The correct approach to determine the contribution of each umbel level is by the number of seeds it contains. However, it is clearly impractical to count all the seeds produced by each umbel level from each experimental plot, so an approximation is required. The easiest way to determine this would be to take a representative seed sample, count the number of seeds it contains, weight it, calculate the average weight of a single seed then multiply the weight of the remaining seed sample to give an estimation of the number of seeds it contains. This is in effect a thousand seed weight (TSW) test (ISTA, 2006). However, the number of seeds is only an estimate, which is problematic as the other measurements of seed quality are also estimates. Multiplying two estimates together means that the result will have even more statistical variation than the component values. Further, it is very difficult, if at all possible, to make a valid statistical comparison of such values.

The alternative of recombining seed from separate umbel levels to recreate the bulk seed, lot is also problematic. Unless the number of seeds produced by each umbel level is exactly the same, withdrawing a set number of seed for testing will alter the

proportion that the remaining seed would contribute to the bulk seed lot. For example, if the secondary umbel level produces 10,000 seeds and the tertiary umbel level produces 500, of the combined 10,500 seeds the secondary umbel level represents 95% and the tertiary umbel level 5%. Two hundred seeds are removed from each umbel order seed lot for testing. The remaining seeds of the secondary umbel level now constitute 97% of a combined 10,100 seed lot and the tertiary umbel level 3%. To preserve proportionality an equal proportion of number of seeds would have to be removed from each seed lot, however, for large numbers of seeds counting them all will not be practical so an approximation would have to be used, i.e., TSW. However, this returns to the problem of multiplying two approximate values.

Even collecting and cleaning each umbel order separately, only undertaking tests that do not destroy the seed, e.g., TSW, and returning all collected seed back to the bulk seed lot is still problematic. For example, if a tiny quantity of small seed from the tertiary umbel level is processed on its own, more seed may be removed by the cleaning process, than if it had been processed with a large quantity of other larger seed that progressed though the cleaning equipment in a greater volume. Therefore, a bulk seed lot where all umbels were processed and cleaned as one may have different properties to the same bulk seed lot, where umbels were processed and cleaned separately, and then recombined.

If these arguments are correct the only way to accurately determine if increasing crop density improves the seed quality of commercial seed lots is to harvest and clean the seed in bulk, as is undertaken by commercial growers, and measure the properties of the bulk seed lot. It is recognised that this goes against the scientific propensity to wish to determine the underlying mechanisms and contribution that each umbel level makes to the bulk seed lot, and the need for sufficient data for publication. However, it is also vital that research that endeavours to be of benefit to the commercial seed industry, should produce results that are applicable to commercial practice. Separation of umbel levels for testing means that the results are difficult to translate into rigorous recommendations for industry.

3.5.2. Role of fungal pathogens

There is no mention in the papers of Jacobsohn & Globerson (1980) and Gray (1981) of fungal foliar pathogens, and it is possible they may not have been a problem in 1980. However, fungal pathogens are now a major problem (Farrar *et al.*, 2004) and these results show that *A. radicina* infestation increased with increasing densities (section

3.3.7) which is a significant seed health problem. It is possible that the benefits of increased seed quality and yield as a result of increased crop density may be overshadowed by concerns about decreasing seed health as density increases.

3.5.3. Carrot seed crop density

A number of the results presented here and by other authors (Noland *et al.*, 1988; Oliva *et al.*, 1988) indicate that 20 plants m^{-2} appears to be the inflection point of a number of the density response curves. This inflection may indicate a change from increasing to diminishing marginal returns as a result of increasing crop density, and that the marginal physical product has reached a maximum. This alone is clearly not a reason to claim that 20 plants m^{-2} is an optimum density as it is based only on one variable input i.e., seed. Other variable inputs needed to grow a carrot seed crop, e.g., fertiliser, equipment costs, labour are likely to account for a march larger proportion of the total variable costs of production than the seed, so a detailed economic analysis of all variable factors of production would be required. However, 20 plants m^{-2} also appears to be the density at which negative effects of inter-plant competition start to manifest themselves, particularly increasing pathogen infestation levels.

In conclusion, the hypothesis that increasing carrot density will increase seed quality is generally supported by these results. However, carrots are clearly a crop that responds to environmental variables in a complex manner. To correctly test the hypothesis, analysis of seed quality must be undertaken on seed produced by harvesting seed from whole plants, not individual umbel levels. As fungal pathogen infestation is now a critical issue for seed production these results indicate that 20 plants m^{-2} is a suitable planting density for carrot seed production.

Unfortunately much of the research conducted by seed production companies, especially that which is of competitive value, such as seed production, is not published. In it therefore interesting that Bejo Zaden B.V., who are the largest global producer of carrot seed, choose 20 plants m^{-2} as their preferred production density due to increased fungal pathogen infestation levels at higher densities (Fred van de Crommert pers. comm. 2006).

Chapter 4. Thermal weeding

4.1. Review of thermal weeding technology and development of a direct-fired steam weeder prototype

Flame weeding is an important and long standing weed management technology for organic growers. The first liquid-fuel flame 'cultivator' was patented in 1852, but, it was not until 1940 that reliable gas phase burners were developed (Atkinson, 1995). The basic design of most flame weeders has changed little from the first machines. Refinements include more efficient burners, hoods to retain the hot exhaust gases close to the ground for longer periods of time and optimised burner angles (de Rooy, 1992; Ascard, 1998b). However, the process is still highly energy inefficient as considerable amounts of energy are used to kill small amounts of weed biomass. This is illustrated in the following hypothetical example. A flame weeder's LPG consumption is 80 kg hectare^{-1} which produces 4000 MJ hectare^{-1}. A mixed sample of the aerial parts of 100 weeds, at the two true leaf stage, from a range of species had a moisture content of 87% and fresh weight of 4.61 g. Therefore, the specific heat of the weeds was assumed to be the same as that of water at 4.18 kJ kg^{-1}. The rise in temperature of the weeds during treatment is taken to be 60°C. Multiplying the weed population, individual weed weight, specific heat and temperature rise gives the amount of heat required to treat the weeds, which when divided by the energy produced by the flame weeder gives its efficiency. Table 4.1 shows a sensitivity analysis for the efficiency of a flame weeder for a range of weed weights and populations, which shows that even with high weed populations, flame weeding is an energy inefficient process and with low populations, it is very inefficient.

Table 4.1 Sensitivity analysis of the percentage efficiency of a flame weeder for a range of weed weights and populations.

		Weight of individual weed (grams)				
		0.01	**0.03**	**0.05**	**0.07**	**0.09**
Number of weeds m^{-2}	**100**	0.06	0.19	0.31	0.44	0.56
	300	0.19	0.56	0.94	1.32	1.69
	500	0.31	0.94	1.57	2.19	2.82
	700	0.44	1.32	2.19	3.07	3.95
	900	0.56	1.69	2.82	3.95	5.08

4.1.1. Alternative thermal weeding techniques

Considering that a fundamental concern of modern organic agriculture is environmental sustainability (DARCOF, 2000; Luttikholt, 2004) the use of non-renewable fossil fuels

in such an inefficient manner is highly questionable. There is a need to improve the efficiency of thermal weeders and to use renewable rather than fossil fuel sources. This has spurred much research into alternatives to flame weeding. For example, the use of ultraviolet (UV) light to kill weeds has been investigated in Denmark and while patents were granted in 1996 the technique has not been commercialised (Fox 1996). Microwave radiation has also been researched (Diprose & Benson, 1984b; Vela-Múzquiz, 1984; Zanche *et al.*, 2003; Sartorato *et al.*, 2006) but has not resulted in any practical machinery. The use of lasers to cut weed stems has been investigated (Heisel *et al.*, 2002), electrocution has been studied (Diprose & Benson, 1984b, 1984a; Vigneault *et al.*, 1990) as has freezing using liquid nitrogen and carbon dioxide snow (Fergedal, 1993).

All these approaches suffer from a range of problems that make them uneconomic or impractical. UV light, microwaves, lasers and high voltage electricity are all inherently dangerous and weeders using such approaches would need robust safety systems to protect operators and bystanders. UV, electric discharge and microwave techniques need considerable quantities of electricity, which is problematic as tractors have only a limited 12-volt DC supply. This requires the use of power-take-off (PTO) powered, or stand alone, electrical generators, which has a significant impact on the efficiency of the total system (multiple energy conversions with losses at every stage). The total energy efficiency of a microwave weeding system was calculated to be 24% based on the efficiencies of the magnetron at 75%, electrical generator at 90%, and diesel engine at 35% (Sartorato *et al.*, 2006). In addition to these losses, soil absorbs microwaves, with 62% lost to the ground (Amista, 2002). To gain satisfactory weed control with microwaves it was calculated that up to 3.4 t ha^{-1} of diesel would be required (Sartorato *et al.*, 2006). Both freezing and electrocution are also impractical because of high energy use (Diprose *et al.*, 1980; Fergedal, 1993) and the lasers are effective only when plants are within 1-2 cm of the laser's focal plane, a level of steering accuracy which is beyond even the best computer-vision guidance systems (Pullen & Cowell, 1997; 2000, P. Garford, F. Poulsen & P. Skjølt, pers. comm. 2003).

A 'low-tech' alternative to the flame weeder, which does not suffer from the problems associated with the above complex technology, is the use of a curved-linear Fresnel lens that focuses sunlight into a narrow strip which heats weeds and soil surface. It is effective at killing weed seedlings and seeds on and in soil (Johnson *et al.*, 1989; Johnson *et al.*, 1990). However, its practical use is restricted to climates with

predictable and regular strong sunlight, particularly if its use is required just prior to crop emergence when timing is critical.

Despite all the research into alternatives, flame weeders are currently still the most effective, economic and practical means of thermal weed control. However, their inefficiency and high use of fosil fuels must be addressed if their use in organic agriculture is to remain tenable.

4.1.1.1. Inherent problems of flame weeders

The low efficiency of flame weeders is partly due to using air as the means of transmitting energy to the plant. Air, while a good insulator due to its low specific heat, is a poor conductor. To overcome this problem, infrared thermal weeders were developed which transmit their heat to plants by radiation rather than conduction. This is achieved by burning LPG in ceramic elements mounted under a hood. However, comparisons of the two techniques have shown that in practice, infrared is no better than flame (Ascard, 1998a). Infrared also suffers from two key drawbacks: (1) The ceramic elements are fragile, which makes them less suitable for use in tractor mounted equipment, and (2) The shading effect, whereby smaller weeds are protected from the radiation by larger ones and/or upper leaves protect the lower leaves and stem. This was demonstrated in an experiment where infrared was more effective than flame at the cotyledon stage but less so for larger plants (Ascard, 1998a).

The use of LPG as a fuel also has other drawbacks. A key one is that it needs to be vaporised from its liquid state before it can be burnt. There are two means to achieve this: by vaporising in the bottle, commonly called ‘gas phase’, or in the burner called ‘liquid phase’. Gas phase withdrawal cools the bottle, which, if there is insufficient volume of LPG and/or the air temperature is too low, results in a pressure reduction within the bottle, which can fall below the pressure required by the burner. A visual indication of this cooling is condensation, and in extreme cases ice, on the outside of the LPG containers. Insufficient pressure can result in it being impossible to use all the LPG in the bottle. If bottles that suffer from this problem are refilled without completely emptying them, the proportion of butane in the bottle increases. This is due to the different vaporisation temperatures of propane (-42°C) and butane (-2°C) so that when the bottles are cold more propane vaporises than butane. As the butane content increases the problems of pressure reduction and inability to use all the LPG in the bottles worsens, resulting in a compounding effect. One solution is to increase the amount of LPG supplying the burners, e.g., have more bottles, but this increases both

cost and weight. Alternatively the bottles could be heated, an obvious source of which is the heat from the flame weeder. However, unless carefully regulated this would be dangerous, as it would be possible to overheat the bottles. Safer approaches such as putting the bottles in a water bath and heating the water adds to complication, cost and weight.

The use of liquid phase nozzles addresses all of these problems, in that the LPG is withdrawn from the container in liquid form, so the cooling effect is insignificant, resulting in no issues with reduced pressure or incomplete emptying of LPG containers. Liquid phase take off is commonly used in applications where the amount of gas used is large in relation to the amount stored, which is common in mobile applications such as forklift trucks. However, the liquid LPG needs to be vaporised before it can be burnt in the nozzles. This is commonly achieved by exposing a length of the delivery pipe to the flame to provide the energy to vaporise the gas, either in a dedicated device or more frequently as part of the burner assembly. However, there are few industrial applications for liquid phase LPG burner nozzles of the energy output required in flame weeders, so they are uncommon. This has resulted in the flame weeding companies that make liquid phase flame weeders designing their own nozzles. There are a limited number of flame weeders that do not preheat the liquid LPG in the nozzle and instead spray liquid LPG from the nozzle which is then burnt e.g., Red Dragon alfalfa field flamers (Flame Engineering, Inc. LaCrosse, Kansas USA). This is a less efficient method of using LPG. It significantly limits the potential to use enclosed hoods as the liquid LPG spray needs to be surrounded by large quantities of air to ensure combustion, making such designs more inefficient.

4.1.1.2. The advantages of steam over hot air for energy transfer to plants

Andreas Bertram (unpublished data) using a laboratory based hot air tunnel, showed that steam/air mixtures were more efficient at transferring heat to an artificial plant leaf than was hot air alone, and argued that to make a significant advance in the effectiveness of thermal weeders, flame weeders should be superseded by steam weeders. A steam/air mixture is more efficient at heat transfer due to the very large specific heat of condensation/vaporisation of water (2260 kJ kg^{-1}). In contrast, the specific heat of dry air is 1.0 kJ kg $°C^{-1}$ and water 4.18 kJ kg $°C^{-1}$. This means that condensing steam releases a large amount of energy into the plant's tissues, compared with either hot air or hot water, making it much more effective. Steam has the further advantage in that its lower temperature and high moisture prevents plants from losing

heat via transpiration after treatment, while there is significant transpirative cooling after flame weeding (Sirvydas *et al.*, 2002).

4.1.1.3. Current steam and hot water weeders

The most common method for producing steam is the pressurised boiler. The basic design of these comprises a combustion chamber, vented to the atmosphere by a chimney and a vessel containing water, which is heated by the fire in the combustion chamber. Transfer of heat to the water is less than 100% efficient because part of the heat produced is lost through the chimney along with the exhaust gasses. To produce steam efficiently and enable it to be transported along pipes, the vessel needs to be pressurised, which introduces the danger of it exploding. This requires a range of safety devices and in many countries, legislative compliance. In addition, to ensure efficient energy transfer from the heat source to the water, the vessel requires a large surface area. All these factors add to the cost, complexity and weight of the boiler, making it less suitable for being mounted on a tractor. Despite these problems, researchers and commercial operators have made, or tested the potential, of steam and hot water weeders using pressurised boilers.

The Waipuna system (Waipuna Products Limited, Auckland, New Zealand) which produces hot water, was initially trialled for horticultural use, but due to the large volumes of water required, it was impractical. It is now used for weed control and cleaning in urban areas where it is carried on a heavy goods vehicle. The effectiveness is also low, which led to the development of a polysaccharide foaming agent derived from plants, which increases the length of time weeds experience raised temperatures. In Australia, Steamwand International Pty. Ltd. (New South Wales, Australia) have developed a steam application system based on a 90 kW steam boiler which is intended for municipal, landscape and intrarow weed control in perennial crops, e.g., grapes (*Vitis vinifera* L.), with the steam applied via high pressure hoses to assorted applicator heads. A pressurised boiler steam weeder has been developed at the Department of Engineering, Nova Scotia Agricultural College, Canada and has been used for comparative trials (Rifai *et al.*, 2003). Another machine has been developed and trialled by the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) (Kolberg & Wiles, 2002) and a machine based around a 140 L kettle-drum has been built in Slovenia (Leskosek *et al.*, 2003). The machines used for research were stationary boilers placed on trailers, and are unsuitable for commercial use. The

Waipuna and Steamwand machines also use boilers designed for static use, and use 240v AC electricity, requiring the use of a generator, adding to cost and complexity.

There are also two commercial steam weeders which are based on a modified pressurised boiler design. The 'VaporJet H1200®' developed and produced by Thermal Options N.Z. Ltd in New Zealand, and the 'Atarus Stinger®' developed by Origin Energy Pty. Ltd., and produced by D.J. Batchen Pty. Ltd in Australia (see Appendix A: Illustrations of the Atarus Stinger® and VaporJet H1200® steam weeders). The VaporJet® uses an 'EcoFlam Minor 12' diesel burner (EcoFlam Industries, Italy) as the heat source. A heat exchanger is made from a spiral of Spirex® pipe (Vaportec Ltd, New Zealand). Spirex® pipe is a metal tube with a helical crimp, which considerably increases its surface area per meter length. The EcoFlam burner flame passes through the spiral of Spirex® pipe, superheating the water pumped through it. At the centre of the coil is a 'Quad Track' nozzle from which water and steam escape in the same direction as the flame and are mixed with the hot exhaust gasses. The burner outlet, Spirex® coil and steam are contained within a stainless steel duct in the shape of a flattened cone that widens away from the burner. The VaporJet® has been designed for controlling intrarow weeds in perennial woody crops, such as top fruit and vines.

The Atarus Stinger® uses separate propane and butane supplies to fuel customised gas burners. These fire down twin stainless steel pipes (approx 250 mm in diameter and 1300 mm in length). Around the outside of the lower third of these is coiled copper pipe through which water is pumped before being injected as steam into the lower parts of the pipes, where it mixes with the combustion gasses from the burners. The Atarus Stinger® is also designed for controlling intrarow weeds in perennial woody crops.

These machines are identical in their basic design; both use a burner to superheat water in a pipe, then inject the resulting steam and/or water spray into the hot combustion gases. To an extent these are pressurised steam boilers, but considerably reconfigured, e.g., a single pipe replaces the pressure vessel. The key difference is that the combustion gasses, rather than being vented to the atmosphere are mixed with the steam/water vapour and applied to the weeds. This improves their efficiency and simplifies their design, making them more practical for agricultural use.

4.1.2. Direct-fired steam

An alternative steam generation method that avoids the use of pressure vessels or pipes is 'direct-fired steam'. In this process, fuel is burnt in a combustion chamber, then cold water is injected into the hot exhaust gases as they exit the combustion chamber,

vaporising it. An example of this type of boiler is the "Johnson CurePak" (Johnson Gas Appliances Co., Iowa, USA). However, existing direct-fired steam boilers are unsuitable for agricultural use for a range of reasons, including their size and that they rely on a long delivery pipe run and high operating pressures to ensure water vaporisation. To be practical for agricultural use, a direct-fired steam weeder needs to boil the water into steam without long runs of pipe or high pressure. This requires that the transfer of heat from the combustion gasses to water is as efficient as possible. In a conventional pressurised boiler the surface area of the interface between the water and hot gasses is maximised to improve heat transfer. In practice, it is achieved by having long lengths of small diameter pipes and/or the addition of fins, crimps etc to the pipes. The Atarus Stinger® uses the former approach and the VaporJet's® Spirex® coil is an example of the latter. However, despite the pipes having large surface areas through which to transfer heat, they are small compared with the surface area of a fine spray of water droplets, i.e., the most effective means of transferring heat from exhaust gases to water is by spraying the water as a fine mist or fog into them. This is the basis for the direct-fired steam weeder.

4.1.3. Development of a prototype direct-fired steam weeder

The key idea for the direct-fired steam weeder was conceived after reviewing the designs of the VaporJet® and Atarus Stinger®. It was realised that the heat exchangers used in both machines would be redundant if water was sprayed as fine droplets directly into the exhaust gasses from a suitable burner. This was because the water would have a considerably larger surface area than the heat exchanger and would therefore transfer heat from the exhaust to the water more effectively while at the same time eliminating the complex and potentially dangerous heat exchanger. However, the author lacked the necessary expertise to calculate the combustion and steam parameters and required dimensions of the boiler so Paul Mesman (Canesis Network Ltd, Lincoln, New Zealand) was contracted to undertake this work[2] The author selected the Ecoflam Minor 30 diesel oil burner as having a suitable power output. Based on the burner's performance, Paul calculated the dimensions of the combustion chamber, water injection duct, steam distribution duct, water consumption and selected the initial water nozzle (a 'WhirlJet®' 'E 5' (Spraying Systems Co. Wheaton, Illinois USA)) as detailed in section 4.1.3.1 and Figures 4.1 and 4.2. The combustion chamber was constructed by Morrow Engineering Co. Ltd., Christchurch, Canterbury according to Paul's design.

[2] Paul Mesman was funded by a 'TechNet' expert access grant to Southern Growers Ltd., Canterbury, New Zealand from Technology New Zealand / The Foundation for Research, Science and Technology.

Under the author's direction and overall design the rest of the weeder was constructed at Geoff Hampton Engineering Ltd., Southbridge, Canterbury with the detail of construction left to the engineers. The author designed the overall size of the hood, the need for adjustable internal height, front and rear flaps, chimney, skids and parallelogram mounting including ridged front arms and chain back-linkages, and specified the volumes of the water and diesel tanks. The author also reconfigured the original water injection duct and steam distribution duct designs into the T shape configuration where the water injection duct joins the steam distribution duct in the centre of the long side. The WhirlJet® nozzle was placed at the centre of the long axis of the steam distribution duct where it joined the combustion chamber such that the cone of spray was directed away from the combustion chamber. The author considered that it produced a relatively solid disk of water rather than a fine hollow cone spray, with the disk of water exiting the nozzle at such a velocity and density that there was insufficient time for the combustion gasses to vaporise all the water before it contacted the sides of the duct. Once this happened the water could not be vaporised and exited the weeder in a liquid state which decreased the machine's performance. The author, after consulting with representatives of Spraying Systems Co., replaced the WhirlJet® nozzle with TX small capacity hollow cone nozzles (Spraying Systems Co.), which increased the amount of water being vaporised.

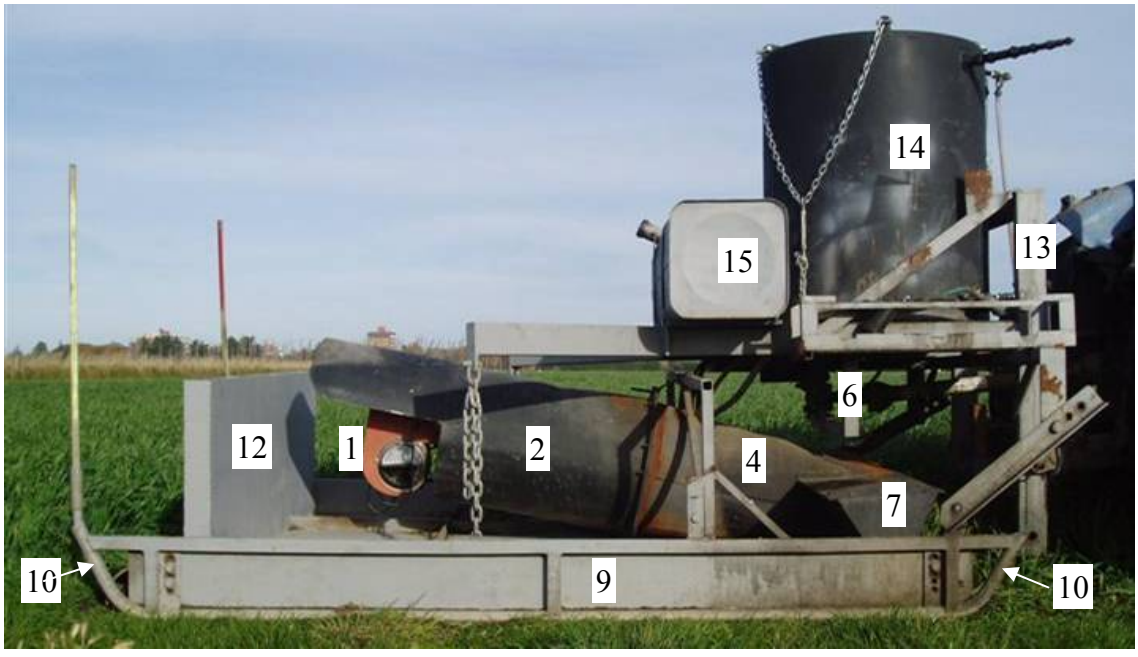

Figure 4.1. Prototype direct-fired steam weeder. parts: (1) EcoFlam burner, (2) combustion chamber, (4) water injection duct, (6) PTO water pump, (7) steam distribution duct, (9) hood, (10) front and rear skids, (12) chimney, (13) tool frame, (14) water tank, (15) diesel tank.

The weeder (Figures 4.1 and 4.2) is powered by the Ecoflam burner (1) with an output of 266 kW and fuel consumption of 20.8 kg diesel h^{-1}. The burner is mounted in the

centre of one end of a cylindrical combustion chamber (2) made of 3 mm thick mild steel plate with a diameter of 500 mm, length of 700 mm and lined with 50 mm 'Kaowool' (3) (Morganite Ceramic Fibres Pty. Ltd. Australia) ceramic fibre insulation, giving an internal diameter of 400 mm. The end of the combustion cylinder opposite the burner opens into a cylindrical water injection duct (4) that tapers from 500 mm to 300 mm internal diameter; it is un-insulated and made from 2 mm galvanised steel plate. At the point of transition between the combustion chamber and the water injection duct are three, brass, size ten, TX small capacity hollow cone nozzles (5) spaced 200 mm apart, positioned symmetrically around the long axis of the combustion chamber/water injection duct and spraying away from the combustion chamber. An AR 202 PTO pump (6) (Annovi Reverberi S.p.A., Modena, Italy) supplies water at 10 bar to the nozzles which have a combined flow rate of 3.5 L min^{-1}.

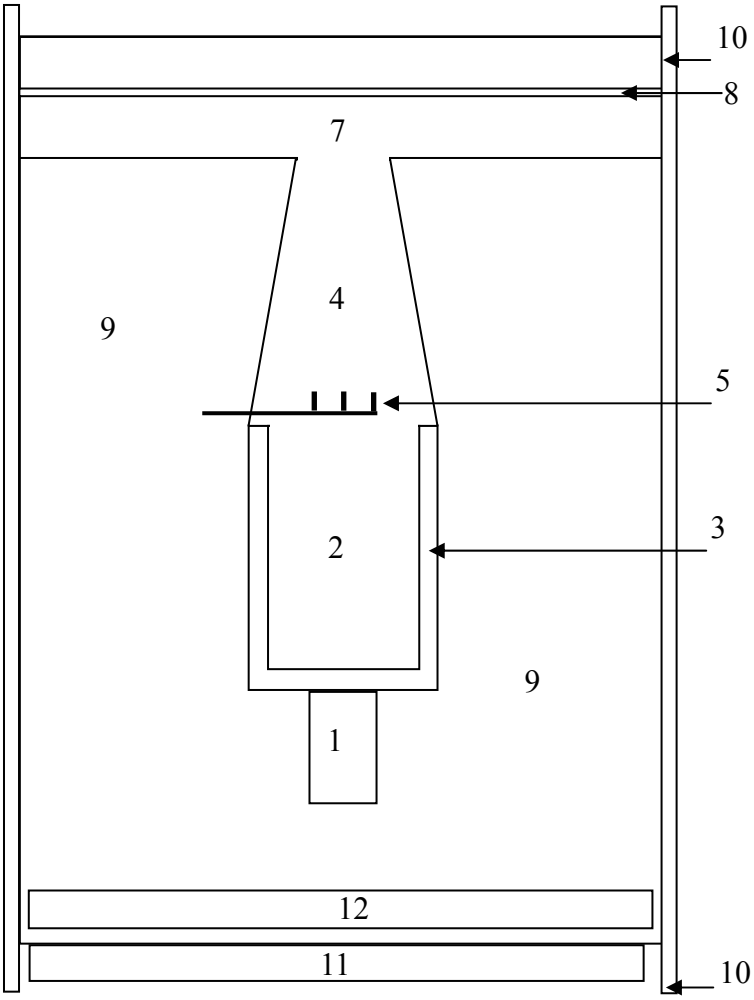

Figure 4.2. Plan view of the prototype direct-fired steam weeder's hood, combustion chamber and steam ducts. parts: (1) EcoFlam burner, (2) combustion chamber, (3) ceramic fibre insulation, (4) water injection duct, (5) TX hollow cone water nozzles, (7) steam distribution duct, (8) steam slot, (9) hood, (10) front and rear skids, (11) rear flap, (12) chimney.

The narrow end of the water injection duct joins at 90° to the centre of a second (steam distribution) duct (7) which has a triangular cross section with all sides being 320 mm

and having a length of 1700 mm. One corner of the steam distribution duct points downwards and has been cut off to make a slot (8) 15 mm wide along the full length of the duct. The burner, combustion chamber, water injection duct and steam distribution duct are mounted on top of a hood (9) into which the steam is introduced at the front, via the slot in the steam distribution duct. The hood is 2.4 m long, 1.750 m wide (internal) and the internal height can be adjusted from 100 mm to 250 mm. It is made from a mixture of mild steel structural parts and 2 mm galvanised steel sheet, and has two skids (10) on either side made from round pipe turned up at the front and back. It has flaps at the front (3 mm mild steel plate) and the back (11) (reinforced rubber) and a chimney (12) 100 mm across by 1700 mm wide and 500 mm high on the top of the hood at the rear. The hood assembly is slung under a tool frame (13) on which are mounted a 500 L water (14) and 150 L diesel tank (15) and an electrical generator (not shown), which supplies 240v AC power to the burner. In use, the hood slides over the ground while the tool frame is supported by the tractor's three-point linkage.

Inadequate water flow to the nozzles will result in insufficient cooling, leading to overheating. A pressure switch is therefore connected to the water supply pipe between the pump and nozzles and set up such that if there is insufficient water pressure, the flame is disabled.

4.1.3.1. Design parameters

N.B. This section has been provided by Paul Mesman of Canesis Network Ltd, Lincoln, New Zealand.

The diesel parameters listed in Table 4.2 were used to calculate the theoretical fuel requirement and combustion gas composition and flow. Complete combustion was assumed with only carbon dioxide and water being produced. The other components of the combustion gas are principally nitrogen and excess oxygen from the inlet air. The gas temperature was obtained from an energy balance using the enthalpy (heat content) of the gas stream, which was calculated by integrating the specific heat functions for each gas component and adding the latent heat for the water vapour.

In the steam weeder, the combustion gases are cooled by evaporation of injected water. The amount of water required to cool the gas to various temperatures is shown in Table 4.3, along with properties of the gas and steam mixture. For example, injecting 272 L h^{-1}, or 4.5 L min^{-1}, gives steam at 200°C.

Table 4.2 Combustion parameters for the direct-fired steam weeder.

Parameter	Value or Detail
Heat input	250 kW
Burner	Ecoflam Minor 30
Fuel	Diesel (transportation)
Higher heating value (gross calorific value)	46.0 MJ kg^{-1}
Density	822 kg m-3
Carbon/hydrogen ratio (mass)	6.2
Excess air	20 %
Diesel consumption	19.6 kg h^{-1} (23.8 L h^{-1})
Combustion gas temperature	1840°C

Table 4.3 Details of combustion gas and steam mixture for the direct-fired steam weeder.

	100°C	200°C	300°C	400°C	1840°C
Water injection rate (L h^{-1})	307	272	241	213	0
Density (kg m^{-3})	0.740	0.592	0.496	0.428	0.167
Steam volumetric fraction (%)	62.1	59.5	56.7	54.0	10.7
Steam mass fraction (%)	49.4	46.6	43.8	41.1	6.7
Dew point temperature (°C)	87.2	86.1	84.9	83.6	47.4
Flow rate (m^3 s^{-1})	0.25	0.30	0.34	0.37	0.61

The condensation temperature (the dew point) of the steam is lowered by the other gases mixed with it, and lies in the range of 84 - 86°C (Table 4.3). Therefore, the steam is cooler at the point of high heat transfer with this method of steam generation, compared with pure steam that condenses at 100°C. The volumetric flow rate of the steam and gas mixture is reduced at cooler temperatures even though it contains more steam (Table 4.3).

Three criteria were used to determine the size of the ducting:

- Gas velocity in combustion chamber = 3 – 5 m s^{-1}
- Overall pressure drop suitable for burner
- Pressure drop across the outlet slot significantly larger than that for the distribution duct

The combustion gas velocity was recommended as typical for furnace design and intended to provide time for complete combustion. The burner backpressure equals the overall pressure drop and must be limited to retain the burner capacity. The capacity is reduced at high backpressure because the burner fan cannot provide sufficient combustion air. In the case of the Ecoflam Minor 30 burner, the maximum pressure is 200 Pa for an output of 250kW. The outlet pressure drop across the slot in the boom determines the uniformity of the flow from the distributor. A higher drop compensates for pressure loss along the distribution duct.

For the dimensions detailed above, the combustion gas velocity is 4.8 m s^{-1}. For 200°C, the total pressure drop was estimated at 62 Pa, well within the burner limit. For the distributor, an estimated 90% of the pressure drop was across the outlet slot, so the steam flow should be uniform. In the event that the burner is operated without any cooling water, the backpressure is expected to rise to 72 Pa, still within the limits for the burner.

4.1.4. Advantages and disadvantages of direct-fired steam weeders over flame and pressurised steam weeders

The direct-fired steam boiler is a simple and effective means of generating steam on tractor-mounted equipment. The boilers can be built using readily available equipment, much of it designed for agricultural use, for example, water pumps and spray nozzles. The design is flexible as the amount of energy and steam can be varied widely, e.g., from 100 to 600 kW. Unlike the VaporJet® and Atarus Stinger®, the method of application and steam generation are separate, so that the same steam generator design, by using appropriate steam delivery systems, could be used for intrarow weed control in orchards, for stale seed beds in vegetable production or treating only the drilled intrarow area.

A wide range of fuels can be used to power a direct-fired steam boiler, including diesel. This is a major advantage for farmers because diesel is used in nearly all tractors and is readily available on farm. LPG in comparison is uncommon and has to be stored in pressure vessels, which are expensive. A further advantage is that diesel can be substituted with bio-fuels or even raw vegetable oil, which can be grown on-farm, eliminating the use of fossil fuels to directly power the steamer. This could address the key criticism of thermal weeders; their inefficient use of fossil fuels. It is noted, however, that in a complete lifecycle analysis (Ciambrone, 1997) fossil fuels and other non-renewable resources would be used in the construction and decommissioning of a weeder and the production of the biofuel may also use fossil fuels so it cannot be considered completely environmentally sustainable.

Steam weeders pose a much lower fire risk than do flame weeders. The steam itself, the controlled and contained combustion process, the low oxygen levels of the exhaust gases, and lower temperatures at the point of application mean that it is very difficult to ignite materials with a steam weeder. For example, steam weeders could be used over paper and polyethylene sheet weed mulches. In comparison, flame weeders constitute a

considerable fire risk due to their open flames and they readily burn and melt paper and plastic mulches.

The performance of flame weeders can be considerably reduced by wind. Flame weeders that use open burners without any kind of hood or protective shield are particularly prone to large reductions in effectiveness as wind speeds increase, but even designs that use shields suffer from problems. For example, a temperature drop under the hood of a flame weeder from approximately 700°C to 100°C from a tail wind of 3.5 m s^{-1} and from approximately 700°C to 500°C for a 3.5 m s^{-1} head wind has been demonstrated (Palmer, 1996). To address this problem a range of baffles, chain and air curtains have been used by manufactures. However, they are not completely effective, for example; in the above test the flame weeder had a series of flat metal flaps on the rear of the machine yet effectiveness was still severely diminished (Palmer, 1996). In contrast, for the direct-fired steam weeder, combustion is in a fully enclosed chamber and is unaffected by wind.

The gas burners of flame weeders have to be situated between 100 mm to 400 mm above the ground and at an angle of around 45° to maximise their effectiveness (Ascard, 1998b) which has resulted in many manufacturers making the hoods a similar height. However, a 100mm shield height has been shown to be the most effective (Storeheier, 1994). If it was lower, it increased resistance to the gas flow and if higher, the gases rose to the roof of the hood, allowing cold air introduced at the front of the hood to sink to the soil surface reducing heat transfer to the weeds. Companies such as Hoaf and Rabaud have addressed this problem by integrating the burners into the hood so it can be lower and have the hood slope down from front to back, so that as the exhaust gases cool and reduce in volume they are kept close to the ground. This sloping hood design also reduces the height of the rear of the hood, so reducing the ability of wind to enter the hood from behind, further improving efficiency. To maximise the effectiveness of a protective hood, only combustion products should be introduced under it, i.e., the ingress of cold ‘outside’ air should be prevented. However, many of the burners used in flame weeders draw only a proportion of the air required for combustion through the burner assembly and rely on the presence of oxygen under the hood to complete combustion. Therefore, there is a need for air to enter under the hood to ensure complete combustion, which reduces under-hood temperature and efficiency.

In comparison, the steam weeder hood needs only to be high enough to clear the ground while leaving sufficient space for the steam. This minimises the under-hood volume

and maximises contact between the steam and the plants. The low profile also reduces the size of the front and rear openings, so reducing wind ingress; baffles reduce it further and the fan on the burner and the gas expansion caused by the combustion process create a positive pressure under the hood so that wind has no discernable effect on performance. Also, with the closed combustion chamber, there is no requirement to introduce air under the hood. The chimney on the rear of the hood allows the hot exhaust gases to escape without allowing wind ingress into the hood, because the hot gases rise up and out of the chimney while the wind blows across the top of the chimney, not into it. This approach has also been used in flame weeders with positive results (C Merfield, unpubl. obs.).

4.1.4.1. Advantages of direct-fired steam over alternative steam weeders

Although the heat exchange pipes of the VaporJet® and Atarus Stinger® are open to the atmosphere at their ends and are therefore not closed-pressure vessels, if the water flow in the pipes is insufficient in relation to the heat being transferred to the water, it could result in excessive water heating, causing rapidly increasing pressure which could culminate in the pipes' rupturing, possibly explosively. Safety systems which turn the burners off when there is insufficient water flow are therefore essential. Direct-fired steam has no pressure vessel or pipes, so avoiding this problem.

Water that contains high levels of calcium, magnesium or other metal ion, hydrogen carbonates (bicarbonates) commonly referred to as 'hard' water poses a problem for steam and hot water weeders that heat water within pipes. Heating such water causes the hydrogen carbonates to convert to water, carbon dioxide and insoluble carbonates ($Ca(HCO_3)_2 \rightarrow CO_2 + H_2O + CaCO_3$) the latter of which precipitate out onto the wall of the pipe, commonly called 'limescale'. Even thin layers of limescale considerably reduce heat transfer through the pipe and require their removal or 'softening' of the water used. Direct-fired steam does not have this problem; although limescale may build up on the nozzles, they are easily cleaned and inexpensive if they need to be replaced.

4.1.4.2. Disadvantages of steam over flame weeders

The two key disadvantages of steam weeders compared with flame weeders are the increase in complexity and the large quantities of water consumed. At the most basic a flame weeder consists of a row of manually ignited gas nozzles and therefore has no moving parts nor does it require PTO power. The addition of hoods and other improvements such as a chimney adds nothing to the complexity in terms of moving or

powered parts. In comparison, at minimum a steam weeder requires water and diesel pumps (if not fuelled by LPG), a fan, ignition spark generator, control and safety systems. Most of these contain moving parts and or electronic circuits, they are often complicated and can constitute a considerable proportion of the weeder's cost. The prototype, travelling at 5 kph on 1.5 m beds would use approximately 300 L ha^{-1} of water, which considerably increases the weight of the weeder and requires that water be readily available.

4.2. A comparison of direct-fired steam and flame weeders and implications for plant morphological responses to thermal weeding

4.2.1. Introduction

Two trials were conducted to compare the direct-fired steam weeder with a flame weeder. The first experiment was a dose-response trial using the weeders at forward speeds of 3 to 7 kph. Mustard (*Brassica cretica* Lam.) was used as a surrogate weed. The second compared the effect of the two weeders at a single speed of 3 kph on the survival of 13 plant species, the majority of which are weeds, at five growth stages. These two experiments corresponded to the two main uses of whole-bed thermal weeders described above, i.e., control of small weeds before crop emergence and control of larger weeds by complete defoliation of both crop and weed.

4.2.2. Experiment 1. Survival of mustard seedlings after steam and flame weeding at five speeds.

4.2.2.1. Methods

The trial was a two-factorial, randomised complete block (four blocks) design with energy dose and weeder type as factors. Energy dose was altered by varying the weeders' forward speed. The weeders were the prototype direct-fired steam weeder and the Lincoln University flame weeder (de Rooy, 1992). The flame weeder was based on a Hoaf KBL 1.5 (Hoaf Infrared Technology, Netherlands) but the burners were redesigned with a 23% increase in efficiency (de Rooy, 1992). The burners are positioned along the leading edge of a hood 200 mm high, 1400 mm wide and 1200 mm long. LPG is supplied at 2 bar to the burners, which are angled at 45° from the horizontal and point backwards. The flame base is 160 mm from the ground and the flame extends to the soil surface. The flame weeder used 17.5 kg LPG h^{-1}, which has an energy content of 243 kWh^{-1}. Details of the steam weeder are described in section 4.1.3. Treatment speeds were 3, 4, 5, 6 and 7 kph determined by the tractor's speed

radar which resulted in dose rates of 507, 380, 304, 253, 217 kW ha^{-1} for the steam weeder and 579, 434, 347, 289, 248 kW ha^{-1} for the flame weeder. Four untreated plots served as controls.

The trial was conducted on a stone-free Templeton silt loam situated at Leeston, Canterbury, New Zealand (43°48'148N'', 172°18'064''E). Mustard seed with 99% germination (ISTA, 2006) was sown in a medium to fine tilth on 18 November 2005 (early summer) in 1500 mm wide beds with a Stanhey MkII belt seeder in four, single rows 300 mm apart with an intrarow spacing of 2.5 mm. The beds were then flat rolled to create a smooth soil surface. Sowing depth was 20 mm. Each plot consisted of a 15 m length of bed with a 20 m inter-plot area for adjusting speed and positioning the weeders. Treatments were applied between 06:10 am to 10:20 am, 10 days after sowing when the plants were between the cotyledon and two true leaf stage. The weather at treatment was clear, with no wind and temperature rising from 4 to 9°C. Assessments were made 29 hours after treatment. The prevailing weather during this time was a mix of sunny and cloudy periods with a minimum temperature of 8°C, a maximum of 18°C and zero to approximately 20 kph winds. Weed populations were negligible and the mustard population averaged 34 plants m^{-1} of row. For each plot, the numbers of surviving mustard plants were counted in five randomly selected 50 cm long strips of crop row. The five sub-samples were totalled to create a single measurement for each plot and these values were then divided by the mean of the four control plots to give a percentage survival as a proportion of the control. The controls were assumed to be representative of the populations in treated plots. Analysis was by ANOVA on untransformed data. It was considered improper to transform the data due to the conversion of counts to a proportion of the control and raw data were normally distributed.

4.2.2.2. Results

There was a significant difference ($p<0.001$) between weeder types with a mean mustard survival rate of 40% for steam and 74% for the flame weeder ($LSD_{0.05}$ 12.1). Survival of mustard plants increased with increasing tractor speed ($p<0.001$) ($LSD_{0.05}$ 19.2) (Table 4.4) and there was a significant effect ($p<0.05$) for the interaction of weeder type and speed (Table 4.4), while increasing numbers of plants survived with increasing speed for both machines survival was always lower for the steam weeder.

Table 4.4. The effect of steam and flame weeding at five speeds on the survival rate of mustard plants expressed as a percentage of the control ($p<0.001$, $LSD_{0.05}$ 27.2 for the interaction).

	3 kph	4 kph	5 kph	6 kph	7 kph
Flame weeder	8	71	96	99	99
Steam weeder	0	7	48	66	78

4.2.2.3. Discussion

The large statistical and biological differences demonstrate that the steam weeder was more effective than the flame weeder for controlling mustard between the cotyledon and two-leaf stages. This, in conjunction with the other benefits of the direct fired steam weeder, demonstrates that direct-fired steam weeders can be an effective, practical and economical alternative to flame weeders when used with the stale seed bed technique.

While the theoretical energy output of the machines is reasonably similar, they vary in a range of ways other than heat source; for example, shield length and height are different, both of which can alter effectiveness (de Rooy, 1992). This experiment, therefore, cannot be considered a direct comparison of the use of flame and steam for weed control; rather it is an evaluation of two contrasting weeders. With modifications, the steam weeder could be run without water, allowing a direct comparison of steam and hot air.

There was also a clear dose dependent result, with a rapid decline in the number of plants killed as speed increased for both weeders which is in agreement with other research (Ascard, 1994, 1997; Peruzzi & Raffaelli, 1999; Juroszek *et al.*, 2002).

A problem inherent to flame weeders that have an open hood front is that, as forward speed increases, more air is forced under the hood, which reduces the under-hood temperature. In a laboratory based experiment the temperature measured 300 mm from the front of the hood, where the burner was positioned, decreased from 790°C to 700°C when forward speed increased from 2 to 4 kph (Palmer, 1996). With the speed more than doubling in this experiment it is possible that increased air ingress at higher speeds reduced the effect of the flame weeder in addition to the reduction caused by the lower energy dose.

4.2.3. Experiment 2. Effect of steam and flame weeding on thirteen plant species at five growth stages.

4.2.3.1. Methods

A randomized complete block split-plot design was used with weeder type and plant growth stage as the two factors and growth stage as the sub-plot. The plant species used in this study were *Amaranthus retroflexus* (L.), *Chenopodium album* (L.), *Capsella*

bursa-pastoris (L.), *D. carota*, *Fumaria officinalis* (L.), *Lolium perenne* (L.), *Polygonum aviculare* (L.), *Rumex crispus* (L.), *Spergula arvensis* (L.), *Stellaria media* ((L.) Vill), *Solanum sarrachoides* (Sendtner), *Trifolium repens* (L.) and *Veronica agrestis* (L.). These species, with the exception of *D. carota*, were chosen as they are common springtime weeds of seed-to-seed *D. carota* crops in the Canterbury region of New Zealand (James Smith, pers. comm. 2003). *T. repens* and *L. perenne* are also important crop species, which form the basis of New Zealand pasture but which are considered weeds when they grow in other crops. The 14 plant species were also expected to respond dissimilarly to thermal weeding due to their differing morphologies.

Due to the difficulty of breaking weed seeds' dormancy, it was impossible to combine the different species into a single experiment. Therefore, each species constituted an individual experiment and statistical comparison among species was not possible. The choice of the five growth stages was based on the results of pre-trial thermal weeding of naturally occurring weed stands. The plants were grown outside at the university nursery in a commercial potting mix (80% horticultural bark (0-12mm), 20% pumice (3-6mm), osmocote exact 3-4 month (16-5-9.2), agricultural lime, Hydraflo) in ¾ size polyethylene bags (pots) 7×7×12 cm (width×breadth×height). Five plants were grown in each pot, equating to 2,000 plants m^{-2}, with ten pots for each growth stage (five per weeder).

Fifteen centimetre lengths of plastic pipe, in which the pots fitted tightly, were buried in bare soil, level with the soil surface as shown in Figure 4.3. Each block consisted of ten pipes, arranged in two groups of five, placed in a line across the middle of two adjacent tractor beds. Each pipe therefore constituted one plot, and there were five blocks in total (only three of which are showing in Figure 4.3). Each set of five pipes had a width of 1.08 m which fitted within the flame weeder's 1.4 m wide hood. The pipe's internal depth was adjusted so that the pot's compost was level with the soil surface. The weeders were randomly allocated to one group of pipes in each block. The weeders were then assigned one bed each. Each group of pipes on that bed, which had been allocated to the weeder to be used, was filled with plants, while the other pipes were left empty. One randomly selected pot of each growth stage was allocated to each pipe. Weeders were then driven down their bed to apply the treatment. The weeders were then swapped over, the empty groups of pipes were filled with plants, the treated plants were removed, and the weeders were driven down the other bed. The weeders were

used at a speed of 3 kph as measured by the tractor's radar based speedometer. Three kph was chosen as a speed that was slower than the weeders' usual operating speed, but was one that growers would consider practical on a field scale and it provided the best results in the first trial.

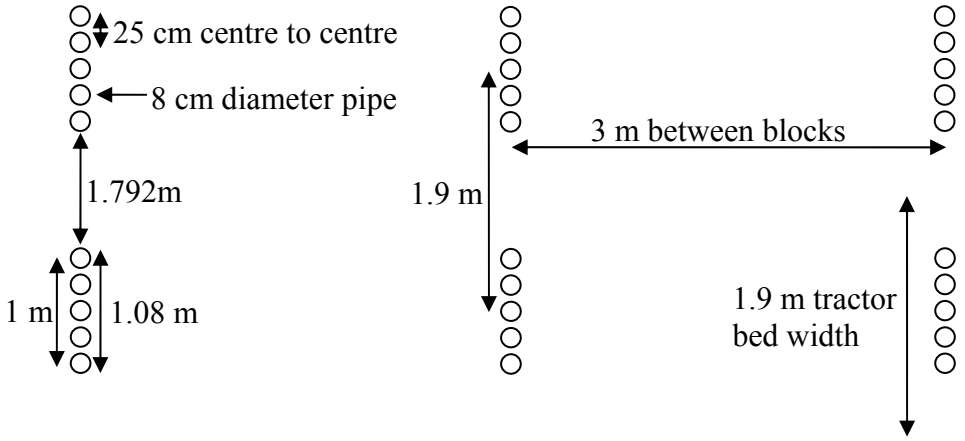

Figure 4.3. Trial layout diagram: small circles represent the pipes sunk into the ground, only three out of five blocks are shown.

After applying the treatments, the plants were returned to the nursery and kept watered. The number of plants that had survived was established on whether plants regrew or died three weeks after treatment. Trials took place between August and March (spring and summer). Treatments were applied before 10:00 am when the wind speed was below 5 kph. In addition, shelterbelts ensured that there was no reduction of the flame weeder's performance due to wind. The site was located at the Biological Husbandry Unit, Lincoln University, Canterbury New Zealand (43°38'998''N, 172°27'380''E). Untransformed data were analysed by ANOVA with a comparison of means by least significant difference. An ANOVA on Arcsine transformed data was completed but produced the same levels of significance, so the transformed analysis was not used. For species which had growth stages with 0% or 100% mortality for both weeders the ANOVA was completed on ARCSIN transformed data with those growth stages excluded. The overall means for the weeders from the re-analysis did not differ in levels of significance from those of the unrestricted untransformed data and were therefore not used.

4.2.3.2. Results

Results from *S. media*, *L. perenne* and *V. agrestis* could not be statistically analysed due to data uniformity. *L. perenne* survived at all growth stages while *S. media* and *V. agrestis* showed high mortality at all stages (Table 4.5). For the other species there was a significant difference in mortality among growth stages ($p<0.001$) with all plants

killed at the cotyledon stage and most at the second growth stage (Table 4.5). However, as plants grew they became more resistant to both flame and steam, although this differed among species. Only for *S. arvensis* (p=0.004) and *C. album* (p=<0.001) did weeder type result in significant differences between overall means with survival rates of 35% for flame and 41% ($LSD_{0.05}$ 5.7) for steam for *C. album* and 43% for flame and 52% for steam for *S. arvensis* ($LSD_{0.05}$ 4.2). There were also statistical differences ($p<0.05$) in plant mortality between machines at individual growth stages. Steam weeding killed more *A. retroflexus* at the fourth growth stage, *D. carota* at the third growth stage and *S. sarrachoides* at the fourth growth stage while flame weeding killed more *T. repens* at the fourth growth stage (Table 4.5).

Table 4.5. Effect of flame and steam weeding at 3 kph on the percent survival of 13 plant species at five growth stages.

	Growth stage and height	Steam	Flame	Significant	$LSD_{0.05}$
A. retroflexus	Cotyledons	0	0	ns	
	Two true leaves 2 cm	0	0	ns	
	Four true leaves 7 cm	52	60	ns	11.9
	Six true leaves 8 cm	96	84	Significant	
	Eight true leaves 11 cm	100	100	ns	
C. album	Cotyledons	0	0	ns	
	Two true leaves 1 cm	0	0	ns	
	Four true leaves 6 cm	28	16	ns	15.0
	Eight true leaves 10 cm	80	72	ns	
	Ten true leaves 14 cm	96	88	ns	
C. bursa-pastoris	Cotyledons	0	0	ns	
	Two true leaves 1 cm	4	0	ns	
	Four true leaves 1 cm	28	32	ns	15.9
	Six true leaves 3 cm	84	84	ns	
	Eight true leaves 4 cm	100	100	ns	
D. carota	Cotyledons	0	0	ns	
	One true leaf 1 cm	0	0	ns	
	Two true leaves 2 cm	12	0	Significant	11.9
	Four true leaves 4 cm	52	56	ns	
	Six true leaves 8 cm	92	96	ns	
F. officinalis	Cotyledons	0	0	ns	
	Two true leaves 1 cm	4	12	ns	
	Four true leaves 1.5 cm	56	56	ns	11.8
	Six true leaves 3 cm	100	92	ns	
	Eight true leaves 5 cm	100	100	ns	

L. perenne	Cotyledon	100	100		
	Two true leaves 1 cm	84	100		
	Four true leaves 6 cm	100	100		n/a
	Eight true leaves 10 cm	100	100		
	Ten true leaves 14 cm	100	100		
P. aviculare	Cotyledon	0	0	ns	
	Two true leaves 2 cm	0	0	ns	
	Four true leaves 6 cm	0	0	ns	15.5
	Eight true leaves 13 cm	20	28	ns	
	Ten true leaves 16 cm	36	28	ns	
R.. crispus	Cotyledons	0	0	ns	
	Two true leaves 1 cm	4	8	ns	
	Four true leaves 3 cm	68	60	ns	16.3
	Six true leaves 7 cm	100	100	ns	
	Eight true leaves 13 cm	100	100	ns	
S. arvensis	Cotyledons	0	0	ns	
	Two true leaves 1 cm	0	0	ns	
	Four true leaves 1.5 cm	68	44	ns	14.3
	Six true leaves 3 cm	92	76	ns	
	Eight true leaves 5 cm	100	96	ns	
S. media	Cotyledons	0	0		
	Four true leaves 6 cm	0	0		
	Eight true leaves 10 cm	0	0		n/a
	Fourteen true leaves 15 cm	0	0		
	Twenty six true leaves 21cm	0	8		
S. sarrachoides	Cotyledons	0	0	ns	
	Two true leaves 1.5 cm	0	4	ns	
	Four true leaves 6 cm	72	60	ns	15.6
	Six true leaves 8 cm	88	72	Significant	
	Eight true leaves 10 cm	100	96	ns	
T. repens	Cotyledons	0	0	ns	
	Two true leaves 1 cm	0	0	ns	
	Four true leaves 2.5 cm	0	4	ns	10.4
	Six true leaves 6 cm	28	40	Significant	
	Ten true leaves 8 cm	92	84	ns	

V. agrestis	Cotyledons	0	0		
	Two true leaves 1 cm	0	0		
	Four true leaves 1.5 cm	0	4		n/a
	Eight true leaves 11 cm	0	8		
	Sixteen true leaves 15 cm	0	8		

4.2.3.3. Discussion

There were large differences in the susceptibility of the different plant species to thermal weeding. For example, *L. perenne* survived treatment at all growth stages while *V. agrestis* had a high mortality rate. Other researchers have also found considerable variation among species. Ascard (1995) found that in a dose response trial on natural weed stands, *Senecio vulgaris* (L.) and *Matricaria discoidea* (DC. synonym *Chamomilla suaveolens* (Pursh) Rydb.) required a propane dose of 35 kg ha^{-1} to obtain 95% reduction in plant numbers, while *C. album* and *S. media* required only 20 kg ha^{-1}. Further, it was impossible to kill *Poa annua* (L.) with a single treatment. This variability has also been seen with other types of thermal weeders, e.g., microwave treatment required 3433 kJ m^{-2} to achieve a 90% reduction in dry weight of *Panicum miliaceum* (L.) compared to 1015 kJ m^{-2} for *Abutilon theophrasti (*Medik.) (Sartorato *et al.*, 2006).

There were also clear biological and statistical differences in susceptibility among different growth stages for each species, except for *L. perenne, S. media*, and *V. agrestis*. Plants were completely susceptible at early growth stages but rapidly gained resistance and often attained 100% survival, showing a sigmoidal response to treatment, which is in agreement with other research (Vanhala & Rahkonen, 1996; Sartorato *et al.*, 2006). However, there has been little discussion of the reasons for the differences between species. Ascard (1995) suggested that upright weeds are more susceptible to flame weeders than prostrate ones and that thin leaves and unprotected growing points increase susceptibility. A deeper understanding is required as to why susceptibility varies among species and growth stages, so cause and effect linkages can be established. This is commonly done with herbicides for which the biochemical cause of the toxic effects is elucidated: the same is required for thermal weeding.

Thermal weeding is a predominately physical process compared to the biochemical action of herbicides. It is possible that the dose-response approach, which has been adopted from herbicide research, is not suitable for developing a predictive theory for thermal weeding. This is because the response of a species to thermal control is not just dependent on the dose of heat but also on how the plant's tissues and morphology

interact with the heat. Also, a species response to thermal weeding can vary based on plant population, weather, growth forms and soil conditions (Hansson, 2002). Even weeders that have similar theoretical heat output based on the energy content of fuel burnt vary markedly in their effectiveness, as demonstrated by the previous experiment (page 76). A dose response curve based on energy value of fuel burnt from one trial could be completely different to a second trial on the same species with different equipment and conditions. This is in contrast to herbicides where, if other variables are fixed, using different spray applicators does not cause large variations in the amount of control achieved. Further, based on results from this experiment, it appears that the variation among weeding events could vary among species, with some species showing highly predictable responses for each growth stage, as seen for *L. perenne* in this study, and others varying as a result of particular site conditions, e.g., *C. album*. These results suggest that plant morphology, the response of plant tissues to heat and how they interact with the heat source may be a better foundation for predicting the effect of thermal weed control.

Growers who use thermal weeding, are mainly using it with the stale seed bed technique and normally wish want to achieve complete weed kill, due to high crop value, often low crop competitiveness and the problem of weeds contaminating the harvest, rather than reducing weed biomass to a given level (Walz, 2004). While it may be valuable for research purposes to determine dose response curves and biomass reduction, such information is of less help to growers whose aim is principally a 100% population decrease. For growers to directly benefit from research, it is important that the effect on weed population is measured as well as biomass.

To effectively kill weeds either the hypocotyl or all the aerial meristems have to be destroyed. The importance of where a plant is cut on the plant's survival was demonstrated by cutting below or above the cotyledons at various stages of development of *Matricaria inodora* L., *C. album* and *P. annua* (Baerveldt & Ascard, 2002). No plants survived when *M. inodora*, *C. album* and *P. annua* were cut through the hypocotyl stem; in contrast, when the epicotyl stem was cut, most survived. The same results was achieved when cutting stems with lasers (Heisel *et al.*, 2001). The need to kill the plant tissues below any point of regrowth, typically the cotyledons, applies equally to thermal weeding.

Response of each species to thermal treatment

The grass *L. perenne* was resistant to thermal weeding at all growth stages. While its foliage was completely destroyed, it rapidly regrew new leaves from its apical meristem. When the plants were small the meristem was protected under the soil, and at later stages it was also surrounded by leaf bases. Similarly, Ascard (1995) found that the grass *P. annua* was highly resistant to thermal weeding. It was shown that drilled onions could be flame weeded with total foliage destruction at the cotyledon (hook) stage with minimal population reduction (Meyer *et al.*, 2002). However, there were significant plant losses when flamed at the two true leaves, while treatment at later stages resulted in minimal population reduction. This is in agreement with research by F. Dastgheib (unpubl. obs.) who compared the flame and steam weeder for thermal weeding of onions at a range of early growth stages. The dicotyledonous *Vicia hirsuta* ((L.) S.F. Gray) and *Vicia tetrasperma* ((L.) Schreb.) were selectively killed in winter rye (*Secale cereale* L.) and winter wheat (*Triticum aestivum* L.) crops with an infrared weeder (Juroszek *et al.*, 2002). The apical meristem of many monocotyledonous seedlings is under the soil surface (Rajan, 2003) and therefore protected from thermal treatment, allowing the plants to regrow, thereby, making them highly resistant to thermal control. Repeat treatments may achieve control by exhausting the plant's underground nutrient reserves.

In contrast, *S. media* and *V. agrestis* were highly susceptible to thermal treatment. However, both are prostrate (Rajan, 2003) which, as Ascard (1995) suggested, should make them less susceptible. Both have thin stems and leaves with unprotected growing points which may increase susceptibility as the heat has to penetrate only a short distance though the plant tissues to kill the meristems. In addition, their leaves are small (less than 5 mm across) so upper leaves offer little protection to lower plant parts. These factors all allow both flames and steam to penetrate to the lowest parts of the plant structure, including the 1-2 cm long and thin hypocotyl, thereby killing the entire plant.

A. retroflexus and *C. album* have an upright growth habit with a single stem that continues to thicken and lignify with age and does not branch until the plant reaches a height of around 10-15 cm (Rajan, 2003). They also have larger thicker leaves, 2-3 cm by 3-6 cm, which in contrast to *S. media* and *V. agrestis* provide more effective protection to lower parts of the plant by blocking the downward movement of the flame or steam. This morphology was reflected in their survival rates. Both species were

highly susceptible at early stages but then rapidly gained resistance, with *A. retroflexus* achieving 100% survival by the eight true leaf stage, while approximately 90% of *C. album* survived at the ten true leaf stage. *A. retroflexus* has thicker, larger leaves and a more robust stem than *C. album*, which might explain the difference. At earlier growth stages, the upright habit allowed the steam or flame to reach the soil surface and the thin hypocotyl (Rajan, 2003), thus killing the plants. However, at later growth stages, the lower stem was protected by the upper leaves and as the stems thickened, they subsumed the axial meristems thereby protecting them. Although the lowest sections of the stem survived and produced new shoots the top of the plant was completely destroyed, which reduced its biomass.

S. sarrachoides also has an upright morphology (Rajan, 2003) but it grew side shoots from the lower leaf axils sooner than *A. retroflexus* and *C. album*. It also appears to be more prostrate when competition is low and its stems are thicker at equivalent growth stages. Despite these differences, which would suggest that it is more resistant, it showed similar susceptibility as *A. retroflexus,* with the earliest growth stages being highly susceptible, but then it rapidly gained resistance.

The upright growth of *C. album, A. retroflexus* and *S. sarrachoides* means they should be controllable at doses higher than used in this experiment, or by split treatments in which the first treatment destroys the upper leaves that protect the lower stem so that the second can reach and kill the critical lower meristems and hypocotyl (Ascard, 1995). All the dicotyledonous weeds tested by Ascard (1995) could be killed up to the four true leaf stage with sufficient heat. *C. album* and *Urtica urens* (L.) could still be killed at the 6-12 true leaf growth stages but even the highest doses could not kill *C. bursa-pastoris*. *C. album* and *U. urens* have an upright growth habit similar to *A. retroflexus* and *S. sarrachoides,* and *C. bursa-pastoris* has a prostrate form (Rajan, 2003). Higher thermal doses enable the heat to reach the lower stem of larger upright plants, penetrate the plant stems, and kill the meristems and/or hypocotyl.

While *F. officinalis*, as opposed to *C. album, A. retroflexus* and *S. sarrachoides*, has small, soft leaves and thin stems, it still became tolerant to thermal treatments quite rapidly. The mature plant has long thin stems that scramble over other vegetation, but the seedling forms a rosette of leaves with the meristems protected by the petioles (Rajan, 2003). In this experiment, the leaves were destroyed by thermal weeding, while the meristems protected by the petiole survived and rapidly produced new leaves. *D. carota* and *C. bursa-pastoris* also form a rosette. Rosette-forming plants are highly

resistant to thermal weeding because both the apical and axillary meristems are protected by petioles that are often thickened. To reach these meristems, heat has to be transferred by conduction through the plant tissues, which is much slower than the convection, condensation or radiation of heat from the weeder to the plant's surface (Sirvydas *et al.*, 2002). *D. carota* has relatively upright leaves and initially the hypocotyl grows above ground, while *C. bursa-pastoris* is prostrate from the earliest growth stages. This difference is reflected in *C. bursa-pastoris* becoming resistant to thermal treatment more rapidly than *D. carota*. In contrast to *C. album* and *U. urens*, for which control at later stages was achieved with higher doses, *C. bursa-pastoris* became immune to thermal treatment once a rosette was formed (Ascard, 1995). Control of rosette-forming plants is, therefore, only possible at the earliest stages of growth.

R. crispus lacks an above ground stem but neither does it form a dense rosette as *C. bursa-pastoris* does. Instead, it has a contractile root that pulls the short stem beneath the soil surface (Rajan, 2003). This adaptation has resulted in it becoming an increasingly problematic weed for organic systems, as mowing or grazing defoliates, but does not kill the weed (Turner *et al.*, 2004). In this experiment, root contraction drawing the apical meristem under the soil surface started at about the four true leaf stage. While the apical meristem was above ground, the plant was vulnerable to treatment, but once protected by the soil it could regrow, even after complete defoliation; consequently, survival rapidly reached 100%. An underground apical meristem is a morphology shared by the monocotyledons (Rajan, 2003). This indicates that, unless treated early, total control of *R. crispus* will be impossible regardless of dose, and that resistance to thermal weeding will be gained rapidly.

R. crispus can also regrow from root fragments (Turner *et al.*, 2004) as can *Taraxacum officinale* (G.H. Weber ex Wiggers) (Hacault & Van Acker, 2006) and *Cirsium arvense* ((L.) Scop.) (Donald, 1990). Thermal weeding can only kill the aerial parts of plants, so species that regenerate from root fragments cannot be killed by a single thermal weeding treatment. Multiple treatments may exhaust the root reserves sufficiently to achieve control. However, thermal treatment is expensive and slow compared to other defoliation techniques such as mowing, which means that it is an unsuitable tool to control this type of weed.

S. arvensis has an unusual morphology; the stem at the base of the cotyledons swells into an unlignified spherical shape from which it produces five to fifteen needle-like, 1-

3 cm long leaves. Branches, that initially look similar to leaves, are also produced from the swollen stem, the ends of which then swell to produce further leaves and branches. The leaves of *S. arvensis* were easily destroyed by thermal treatment; however, by the eight true leaf stage, the swollen stem was sufficiently large to survive thermal treatment. At the earlier growth stages, the hypocotyl stem was above ground and thus susceptible to thermal treatment but, as the plant grew, it contracted, i.e., by the eight leaf stage the swollen stem rested on the soil surface protecting the hypocotyl. Although the swollen stem increased the survival rate, it did not offer the same protection as a leaf rosette. The open structure and thin leaves provided limited protection to the stem as they readily allowed the flame or steam to penetrate the entire plant structure. This indicates that *S. arvensis* should have a response to thermal weeding that is similar to weeds with an upright morphology, i.e., at later growth stages, it should become resistant to lower energy doses while higher doses should kill it.

P. aviculare is a prostrate weed, with a thick, lignified, very short main stem, which produces many thin, side stems that fan out to form a mat. The leaves are spatulate, up to 1 by 3 cm in size and moderately spaced along the stems. This means the growing points in the leaf axils are not well protected and the leaves and prostrate form means all parts of the plant are exposed to the flame or steam. This was confirmed by the results of this study, which showed that *P. aviculare* was highly susceptible to thermal treatment with the majority of plants killed at the ten-leaf stage. The plants that survived all regrew from the lowest leaf axils on the stem, where it was thickest and lignified. During preliminary field tests, the peripheral stems and leaves of larger plants were destroyed when treated at 1 kph, but the thick central stem survived, resulting in rapid regrowth of the lateral stems. Contrary to Ascard's (1995) observations, the prostrate form of *P. aviculare* increased its susceptibility to thermal weeding as it allowed the flame and steam to reach all parts of the plant. In addition, its small thin leaves offered little protection to its axial meristems. Thermal resistance in *P. aviculare* strongly depends on its thickened, lignified stem, which means that mature plants are likely to be resistant to even high heat doses.

T. repens produces stolons that enable it to spread through pasture (Rajan, 2003). Observations of its response to treatment at 1 kph in preliminary tests showed that, while the leaves were easily killed, the partially buried stolons survived and quickly regrew. However, in the trial presented here, the plants became resistant to thermal treatment even before stolons had formed. The plant forms a short, un-lignified 1-3 mm

thick stem close to the ground, from which thin petioles 5-10 mm in length extend with a trifoliate leaf at their ends. This means that at a density of 2,000 plants m^{-2}, there was a complete cover of leaves by the ten-leaf stage. Although the leaves were highly susceptible to thermal treatment and wilted rapidly, they proved an effective barrier to the thermal treatment, as they remained turgid during the short period that the flame or steam curtain passed over them. Their position 8 cm above the ground created a large air gap underneath filled with smaller and shorter leaves, which provided considerable protection for the stems at the soil surface. The plants that were killed, particularly by the flame weeder, were those in the pots facing away from the direction of travel. This may be because the flames, angled at 45° to the horizontal and facing rearwards, could reach the plant's base as they passed over the pots. In contrast, the steam was applied vertically from above so that the leaves could offer protection to all the stems. This suggests that a split treatment with the first aimed at destroying the leaves and the second to kill the stems could achieve total control. This split dose approach varies from the approach suggested by Ascard (1995) who applied two half doses 13 d apart. These results indicate that the split dose would require a standard or higher dose to be effective, and that the second treatment should be completed as soon as the damaged foliage has dried off enough to offer little protection to the remaining parts.

In summary, the key factors determining plant responses to thermal treatment are:

Complete control can be achieved if the thermal treatment can reach and kill the hypocotyl stem. In contrast, if terminal or axillary meristems are covered by the soil or protected within thick stems, if the plant forms a rosette of leaves or can regenerate from root fragments, total control can not be practically achieved by thermal weeding. Canopies of leaves offer considerable protection. However a sufficiently high dose or a split treatment approach can work if the first treatment exposes the stem and allows the second treatment to kill all aerial meristems or the hypocotyl stem. With this approach control is possible but treatment costs will be high.

Targeting of the hypocotyl stem for improved thermal control

The need to kill the hypocotyl stem suggests two approaches to improve thermal control of larger weeds. The first approach uses a 'steam knife' to kill a ring of tissue below the cotyledons thereby killing the entire plant (Cesna *et al.*, 1998; Sirvydas *et al.*, 2002). This should use considerably less steam than would be needed to kill all the foliage. This is similar to the technique of 'ring barking' trees, where a circular cut is made around the base of the tree that is deep enough to sever the phloem, thus starving the

roots of nutrients and eventually killing the tree. Targeting the steam at the hypocotyl by reconfiguring the steam outlet may considerably reduce the amount of steam required. The second approach is to remove the upper parts of the weeds mechanically, as close to the ground as possible, leaving the hypocotyl stem exposed to thermal treatment. As farmers have access to a wide range of mowing equipment, using the faster and lower cost mowing equipment to remove the bulk of the foliage and the slower, more expensive thermal weeder to kill the stem base would be a more practical and economic approach.

Weeders' relative performances

While the previous experiment demonstrated a large statistical and performance based difference between the two weeders across all speeds, there was no significant difference at 3 kph. This is in general agreement with the results of the second experiment, except for the overall means for *S. arvensis* and *C. album* and the individual means for machine and growth stage which showed that the steam weeder achieved greater mortality in three cases and the flame weeder in one case. As a key argument for steam weeders has been their superior performance, the better control of *S. arvensis* and *C. album* by flame and the lack of difference for the majority of the individual means requires explanation. Possible reasons are that the steam curtain has a slower velocity than that of the flame from the LPG burners so the flame's greater velocity may enable it to better penetrate foliage and reach the critical stem base. The vertical direction of the steam curtain means that it hits the top most parts of the plants straight on. For species such as *T. repens*, this is where the densest foliage occurs, which provides an effective barrier to the steam, thereby protecting the lower stems. The burners of the flame weeder are angled at 45°, which means the flame hits the top leaves obliquely and may aid penetration. As noted previously for *T. repens*, it could reach the base of the outer plants when it passed over the pots. Further, it is expected that the steam condenses on to the plant material it first comes in contact with, releasing the majority of its energy into that tissue, while the flame would give up a much smaller proportion of its energy to the first leaves it hits allowing more heat to reach the stem base. Bare earth, as used in this study, may not have been the best surrounding for the pots. Vegetation of a similar height around the pots may create more realistic conditions; however, the plants' varying heights mean that it may be equally confounding. Clearly, these suggestions need to be studied using steam curtains with equal velocity and angle as used by the flame weeder, and the issue of the edge effect needs to be addressed.

Further, at lower speeds the large thermal dose received by plants from flame and steam may obscure the differences in effectiveness between them. For example, if 1 kw is required to kill a given amount of weeds and the weeders produce 20 kw each, the steam weeder transfers 10% to the plants while the flame weeder transfers 1% then the plants will receive 2 kw from the steam weeder and 0.2 kw for the flamer, so the steam weeder should kill more weeds that the flame weeder. If the thermal weeders produce 100 kw, e.g., by lowering their speed, then the steamer transfers 10 kw and the flamer 1 kw so both weeders should kill all the weeds. Therefore the greater effectiveness of steam found in the laboratory studies may be better demonstrated at lower overall doses. Finally, where the meristems are protected within the plants tissues there is a two stage heat transfer process; firstly by convection, condensation or radiation from the heat source to the surface of the plant, then by conduction through the plant tissues. The latter is considerably slower than the former (Sirvydas *et al.*, 2002) and the rate of conduction is likely to be the same regardless of heat source. It is possible that the initial difference in the amount of energy delivered to the plants' surface may be diminished by the slower process of conduction, resulting in a reduced difference between flame and steam.

4.3. Effect of flame weeding carrots at bolting on height, flowering and seed quality

The above study has shown that carrots can survive thermal treatment at comparatively young growth stages. However, it is not clear if thermal weeding can negatively affect a carrot seed crop if it is used at later crop growth stages, especially when the plant moves from vegetative growth into the reproductive phase. An experiment comparing the effect of flame weeding on steckling carrots at the start of bolting and then nine days later was undertaken.

4.3.1. Methods

A randomized complete split block design with four blocks was used. The main plot was flame treatment time and the subplot was cultivar. The cultivars used were Bejo F1 hybrid parent cultivars: A680209 (seed parent) and C680459 (pollen parent). The pollen parent was removed at the end of flowering and only seeds from the seed parent were harvested. The trial was conducted on a Bio-Gro certified organic (Anon., 2001) farm in Ashburton, Canterbury New Zealand (43°53'928''S, 171°49'766''E) on a Templeton Eyre soil type in a commercial carrot seed crop. Plots consisted of two rows, seven meters in length, 60 cm apart, with carrots planted 30 cm apart within the row.

There was a five-meter space between blocks for positioning the tractor and flame weeder. Stecklings were supplied by the farmer from his commercial crop and planted on the 28 August 2003. Plots were maintained by the farmer as part of normal crop husbandry, including irrigation and weed management.

The flame weeder was the Lincoln University flame weeder as described in section 4.1.3. Flaming was conducted at 2 kph which was determined by using a set engine speed and gear ratio, which had been timed over 100 m.

The flame treatments were applied; the first on 18 November, 81 d after transplanting, and the second on 27 November, 90 d after transplanting. There was also an untreated control. Ten plants per plot were marked and their height was recorded at regular intervals from 11 November until 12 February 2004. The ten plants were considered sub-samples and their values were averaged before being analysed by ANOVA. The time from transplanting the stecklings until the first plant in each plot started flowering was recorded. Plots of the seed parent were manually harvested on 16 March, while the pollen parent was discarded. Plants were air dried at room temperature, then manually threshed and de-awned by rubbing between two pieces of Flotex carpet (Bonar Floors Ltd., Derbyshire, England), the bottom piece of which was supported on a flat desk and the top piece was glued to a 40×40 cm piece of plywood. Seeds were then graded using a Westrup seed cleaner (Westrup A/S, Slagelse, Denmark, model LA-LST) with a 4.0 mm round scalping screen, a 1.75 mm slotted top screen and a 0.7 mm slotted bottom screen (400 rpm shaker motor speed). The fan settings were pre-aspiration gate 1, filter valve 1.5, fan outlet valve 10. The seed was then passed through the indented drum section of the Westrup seed cleaner using a 3.0 mm indent.

The yield of cleaned seed from each plot was weighed and sub samples were obtained using a rotary seed divider (Hoffman Manufacturing Co., Oregon, USA, model HMCUD). Eight sub samples were tested for germination (ISTA, 2006) except that rolled towels were used in place of blotters. Vigour and thousand seed weight (TSW) were determined on the same sub samples (ISTA, 2006) with the accelerated ageing test (41°C for 72 hr) used as the vigour test. Analysis was done by ANOVA on untransformed and transformed data when data. The square root transformation was used on flowering date and final height, the natural log transformation was used on yield and TSW and the ARCSINE transformation was used on germination and vigour tests. In most cases data was normally distributed and transformation did not alter the results, so only untransformed data are presented unless transformation was required.

4.3.2. Results

At the time of the first treatment, plants had only just started producing an inflorescence stalk and had an average height of 19 cm, while at the second treatment the inflorescence stalk had started to elongate and the average height was 25 cm. After treatment plant height was decreased by 9 and 10 cm for the first and second treatment, respectively (Figure 4.4). The plants showed a flat sigmoidal growth response with a loss of height after treatment, followed by a more rapid growth phase during December, which then levelled off (Figure 4.4).

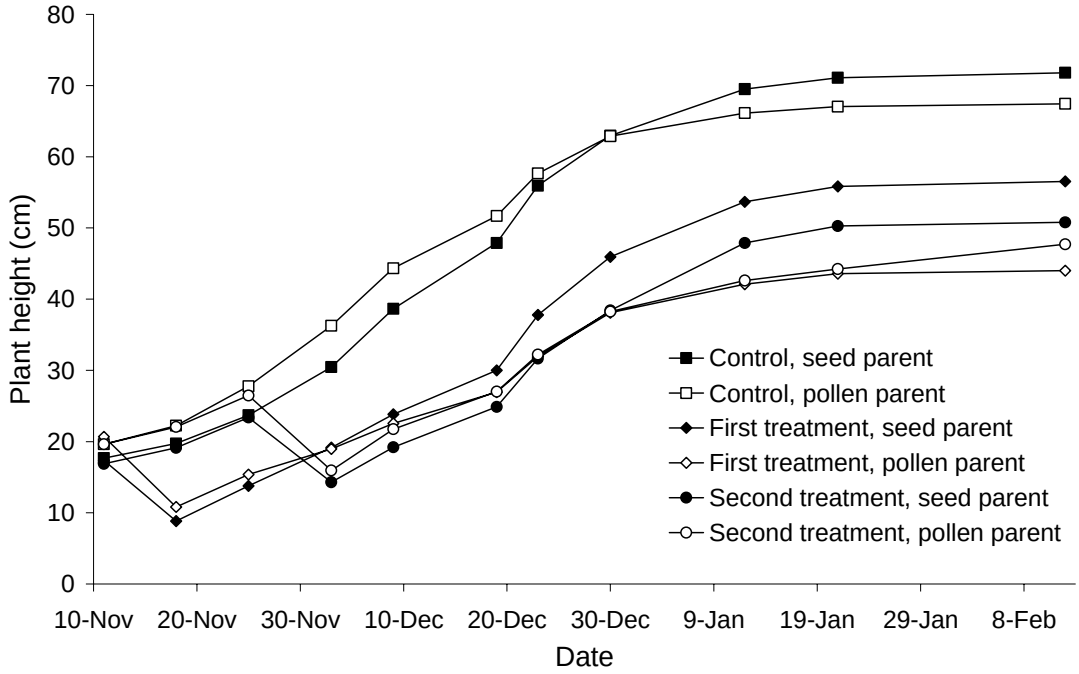

Figure 4.4. Record of plant height over time for control and two flame weeding treatments ($LSD_{0.05}$ 4.8). Flame weeding treatments were carried out on 18 November (first treatment) and 26 November (second treatment).

There was a significant difference between control and treated plants for the start of flowering (Table 4.6).

Table 4.6. Days to flowering, final plant height, yield, TSW, germination and vigour score for early late flame weeding treatments and control (data are untransformed expect where stated).

	Control	1st Treatment	2nd Treatment	$LSD_{0.05}$	p value
Days to flowering	107	115	117	4.6	0.005
Final height (cm)	70	50	49	4.8	<0.001
Yield (log trans.)	5.79	5.20	5.08	0.745	0.061
Yield (grams)	346	182	177	n/a	n/a
TSW (grams)	1.49	1.63	1.61	0.319	0.539
Germination (%)	81	76	68	8.0	0.01
Vigour germ. (%)	32	33	20	16.7	0.192

There was a significant difference among treatments for final plant height (Table 4.6) and between parent cultivar (p= 0.03) with the pollen parent reaching 53 cm and the seed parent 60 ($LSD_{0.05}$ 5.6) but the interaction was not significant (p=0.27). There was

no significant difference in the cleaned seed yield between treatments using log transformed data, although there was a considerable biological difference among treatments (Table 4.6). There was no significant or biological difference for TSW, however, germination was significantly affected by the treatments, while in comparison, there was no significant difference among vigour (Table 4.6).

4.3.3. Discussion

The flame weeding had a clear overall negative impact on the carrots. The flaming created a sizeable check on plant growth and reduction in height from which the plants did not fully recover. This was also reflected in the delay of the start of flowering. Although there was no significance at the 5% level for yield, the control yielded 53% more than the first treatment and was significant at the 6% level. This indicates that it may be a real effect but there was insufficient statistical power to detect the difference at the 5% level. Germination was also negatively affected by the flame treatment with large decreases in germination compared to the control. While a few plants lost their primary umbel, which is known to produce higher quality seed (Jacobsohn & Globerson, 1980), however, it is unlikely this was responsible for the entire reduction. Flaming caused considerable defoliation of the plants which is likely to reduce the photosynthetic resources the plants could devote to seed production, which would be expected to reduce seed yield and quality. Only TSW and vigour showed no statistical or biological differences among treatments. This was somewhat unexpected, especially for the second treatment, considering there were significant differences for other measures of seed quality as a result of flame weeding. However, germination was already low, even for the control, indicating that all the seed was of low quality, which suggests that external factors may have affected the results. One potential reason was aphid infestation; aphids were present on the carrots although populations did not appear high on the living plants. However, once the plants were harvested, and brought indoors to dry, larger than expected numbers of aphids were noted on the floor around the plants. The aphids appeared to be concentrated within the umbels, and it would not be unexpected, if their populations were high enough, they could consume a considerable proportion of the sap flowing to the seeds potentially reducing seed quality. The small biological and lack of statistical differences between the two flame treatments indicate that considerable harm was done even though the carrots had only just started to bolt.

Thermal weeding of crops after establishment is uncommon in weed control in general and flame weeding destroying both weed and crop foliage is even less common than specifically targeting crop stems and intrarow areas with the flame. A comparable study (also discussed on page 84) used an infrared flame weeder to control *V. hirsuta* and *V. tetrasperma* in winter cereals (Juroszek *et al.*, 2002). They found that treatment at early crop stages successfully controlled weeds without reducing crop yields, so that early flame treatment resulted in yield gains *cf*. the untreated controls, while the opposite occurred when treatment was delayed as the crop suffered a considerable growth check.

It would appear that thermal weeding at later crop stages has serious negative impacts on crops and should therefore be avoided. This is expected, as at later stages crop plants are likely to have more biomass, which if destroyed will take longer to re-grow, while the length of time before harvest, in which the lost biomass has to be replaced, is shorter, thereby producing a compounding negative effect.

A better alternative for weed control in carrots at later growth stages could be flaming of the crop base and intrarow area. The inflorescence stem is lignified and should be able to withstand a thermal dose at its base that is sufficient to kill smaller weeds. However, at later crop growth stages weeds would also be larger and also more resistant to treatment, as the results reported in section 4.2.3 show. Thermal weed control at later stages of carrot seed crops therefore appears to be of limited use, and negative impacts on seed quality and yield can be expected, in particular, when the crop foliage is targeted. The results of this study suggest that flame weeding at early growth stages is the most suitable option to control weeds in carrot seed crops, and that treatment at later stages, especially when the inflorescence stem has started to form, should be avoided.

4.4. Chapter Conclusions

The direct-fired steam weeder approach is flexible and has a number of advantages over flame weeders. The amount of energy and steam can be varied widely. Steam could be targeted at specific areas, for example under fruit trees or only in the crop row. Diesel is a convenient fuel for farmers and it could be substituted with renewable bio-fuels, which would address the issue of inefficient use of fossil fuels. Steam weeders pose a low fire risk due to the steam itself, the controlled and contained combustion process, the low oxygen levels of the exhaust gases, and lower temperatures at the point of application. They are also unaffected by wind as combustion is in a fully enclosed

chamber, and by circumventing the need for a pressure vessel, they avoid the dangers and costs associated with them.

While the prototype has demonstrated proof-of-concept there are many areas that need to be improved. Two key issues are: to increase the volume of water vaporised as the 3.5 L min^{-1} is below the calculated optimum; and to optimise hood length as most of the energy is transferred by condensation which occurs within the first 60 cm of the hood (as determined by hood temperature), so a 2.4 m long hood may be excessive.

It is hoped the direct-fired steam weeder described here is a useful starting point for further research into using direct-fired steam weeders for weed control, and for the design of commercially-produced steam weeders.

For use in the stale seed bed technique to kill small weeds the steam weeder is clearly superior to the flame weeder. However, this difference is diminished at lower speeds and with larger plants. The dose-response approach to weed control used in herbicide work may not be the most fruitful approach to predicting plants' responses to thermal weeding: rather a better understanding of how a plant's morphology and the composition of its tissues interact with heat and moisture is needed to enable predictive models, or at least tools for farmers and growers to assess the potential for thermal treatment to reduce weed biomass and or populations.

Further work is clearly required. There is a need to better study the interaction of plant tissues with thermal treatments, including how plant tissue responds to steam and flame, and to determine in more detail the degree of variation among plant species and growth stages. More detailed work is required on how plant morphology affects a plant's response to thermal treatment. There is also scope to improve the steam weeder, particularly as to how the steam is applied to larger plants.

Thermal treatment of whole carrot plants at later grown stages, particularly once bolting has started is likely to result in delayed flowering and reduced yield and seed quality and should therefore be avoided. However, this research has not addressed the issue of the impact of thermal treatment of young carrots on yield and seed quality, particularly overwintered carrot stecklings just prior to commencement of spring growth. This is important because seed producers are starting to use spring thermal treatments to disinfest the crop of both fungal pathogens and aphid species (James Smith pers. comm. 2005). Further, the results presented in section 5.10 indicate this may be a valuable technique in an integrated pathogen control program. Experiments to determine if there

are negative effects on the crop due to late winter or early spring thermal weeding should be established for a range of F1 parent cultivars.

Chapter 5. Fungal pathogen management

5.1. Introduction

Three approaches for managing *A. dauci*, *A. radicina* and *C. carotae* were studied; hot water treatment of seed, using foliar applied biological control agents (BCA) and thermal treatment of small carrots. The hot water experiments also studied the effect pre-treatment seed moisture content had on carrot seed germination and *A. radicina* infestation levels, and compared a logistic curve fitted to daily germination data with total germination, to see if it was a more sensitive means of testing for negative effects on seeds. Testing of BCA used a progression of experiments starting with *in vitro* laboratory studies and finishing with field trials. A range of agar media were tested for their suitability for growing both pathogens and BCA, and also for sporulation of the pathogens. The thermal treatment involved defoliating carrots infested with the pathogens and seeing if the regrowth foliage was infested.

5.2. Hot water treatment of seed

This section studies the effect of hot water treatment and altered seed moisture content on carrot seed that is free of pathogens and on carrot seed with high infestation levels of *A. radicina*.

5.2.1. Experiment 1: Effect of hot water treatment on carrot seed viability in the absence of pathogens

5.2.1.1. Methods

Carrot seeds of an unknown F1 hybrid cultivar, known to be free of the fungal pathogens *A. radicina* and *A. dauci*, were used in a three factorial hot water experiment with factors being initial seed moisture content (SMC) (5, 10, 15 and 20%), duration of treatment (0 (control), 10, 20, 30, 40, 50 and 60 min) and temperature (45, 50 and 55°C). SMC was determined using an internationally standardised method (ISTA, 2006) and was adjusted by either placing seeds in a 30°C oven until the target weight was reached (for 5% SMC) or adding the required amount of sterile distilled water and keeping seeds for 24 h at 5°C (for 10-20% SMC). The amount of water to be added or removed was calculated by the formula in Equation 5.1.

Equation 5.1. Equation to determine the amount of water to be added or 'removed' from seed to achieve target SMC (IWS = initial weight of seed).

$$\text{Change in weight required} = \left(\left(\frac{100 - \text{Initial SMC\%}}{100 - \text{Target SMC\%}} \right) * \text{IWS} \right) - \text{IWS}$$

A sub sample of SMC adjusted seeds were tested (ISTA, 2006) to confirm that the correct SMC had been obtained.

Approximately 400 seeds were contained in stainless steel tea infusers and placed in a hot water bath containing 11 L of water, which was agitated by a shaker plate. After treatment, all seeds were immediately plunged into 15 L of tap water at 15°C for 5 min to cool them rapidly. A germination test was then immediately conducted following a standard method (ISTA, 2006), except the seeds were placed on rolled towels instead of the surface of a blotter. Seeds were incubated in alternating 8 h/30°C light and 16 h/20°C dark cycles. The number of normal and abnormal seedlings (ISTA, 2006) was counted each day for 14 d and dead seeds were counted on day 14.

Data were analysed with ANOVA on arcsine transformed and untransformed data. Germination curves were calculated and fitted to a logistic model (Equation 5.2) (Richard Sedcole, pers. comm. 2003), where y is the number of germinated seeds and x is days. The values γ, β and μ for each curve were analysed with ANOVA. γ is a measure of final germination (number of seeds out of 50), β is the germination rate coefficient and μ is the point of inflexion of the curve, which is a measure of the 'average' time to germination measured in days.

Equation 5.2. Logistic curve formula.

$$y_i = \frac{\gamma}{1 + \exp(-\beta(x_i - \mu))} + \varepsilon_i$$

5.2.1.2. Results

The data were normally distributed and as transformation did not alter the results, the untransformed data are presented. The effect of both treatment temperature and duration on germination was significant ($p<0.001$) with germination decreasing with increasing treatment duration and temperature. There was a significant interaction ($p<0.001$) between the two factors (Table 5.1), in that at 45°C germination did not differ among treatment durations, but at 50°C was reduced after 50 min, and at 55°C after 20 min.

Table 5.1. Effect of duration × temperature on germination percentage ($p<0.001$, $LSD_{0.05}$ 4.3).

	Duration minutes						
	0	**10**	**20**	**30**	**40**	**50**	**60**
45°C	82	84	81	77	81	80	82
50°C	81	81	81	80	80	72	65
55°C	78	77	62	38	19	5	1

The effect of increasing SMC (Table 5.2) was a significant decrease in germination ($p<0.001$), but the reduction was biologically small. There were no significant interactions ($p>0.05$).

Table 5.2. Effect of SMC on germination ($p<0.001$, $LSD_{0.05}$ 1.9).

	SMC %			
	5	**10**	**15**	**20**
Germination %	68	66	66	63

For dead seeds there was a significant difference for treatment duration ($p<0.001$), SMC ($p=0.36$) temperature ($p<0.001$) the duration × temperature interaction ($p<0.001$) and the three way interaction SMC × temperature × duration ($p=0.002$). Increasing duration increased the number of dead seeds (Table 5.3). However, SMC did not affect the percentage of dead seeds (
Table 5.4). For temperature, 55°C had approximately three times the number of dead seeds compared with 45 and 50°C (

Table 5.5).

Table 5.3. Effect of treatment duration (mins) on percentage dead seeds and abnormal seedlings.

	Duration (mins)								
	0	**10**	**20**	**30**	**40**	**50**	**60**	**$LSD_{0.05}$**	**p value**
Dead seeds (%)	6	5	7	8	10	15	22	2.0	<0.001
Abnormal seedlings (%)	5	5	5	3	3	3	3	1.1	<0.001

Table 5.4. Effect of SMC on percentage dead seeds ($p=0.036$, $LSD_{0.05}$ 0.85)

	SMC (%)			
	5	**10**	**15**	**20**
Dead seeds (%)	10	10	9	11

Table 5.5. Effect of treatment temperature (°C) on percentage dead seeds and abnormal seedlings

	Temperature (°C)				
	45	**50**	**55**	**$LSD_{0.05}$**	**p value**
Dead seeds (%)	7	5	18	1.3	<0.001
Abnormal seedlings (%)	5	5	3	0.7	<0.001

The treatment duration × temperature interaction (Table 5.6) also has some ambiguous results with the 45°C treatment showing a trend of increased numbers of dead seeds for longer treatment durations while the hotter 50°C did not. The 55°C treatment had rapidly increasing percentage of dead seeds with increasing duration (Table 5.6).

Table 5.6. Effect of the treatment duration (mins) × temperature (°C) interaction on percentage dead seeds ($p<0.001$, $LSD_{0.05}$ 3.5).

	Duration minutes						
	0	**10**	**20**	**30**	**40**	**50**	**60**
45°C	6	5	5	4	7	9	13
50°C	5	4	5	7	5	5	6
55°C	6	7	11	13	17	30	45

The three way interaction of treatment duration × SMC × temperature (Table 5.10) shows the increased number of dead seeds at 50 and 60 min for the 45°C temperature

and the lack of similar response at 50°C. The 55°C treatment shows much earlier increase in dead seeds.

For abnormal seedlings there were significant differences for treatment duration ($p<0.001$), temperature ($p<0.001$), the duration × SMC interaction ($p<0.001$), and the duration × temperature interaction ($p<0.001$). Increasing duration resulted in a decrease in abnormal seedlings, although the change was only two percentage points and biologically is small (Table 5.3). Similarly increasing treatment temperature caused a small decrease in abnormal seedlings (Table 5.5). The duration × SMC interaction showed a general reduction in abnormal seedlings from the shortest duration and 20% SMC to the longest duration and 5% SMC (Table 5.7). The treatment duration × temperature interaction shows nearly no change at 45°C, a lack of clear pattern at 50°C but a clear decrease at higher temperatures at 55°C (Table 5.8)

Table 5.7. Effect of the treatment duration (mins) × SMC (%) interaction on percentage abnormal seedlings ($p<0.001$, $LSD_{0.05}$ 2.2).

	Duration minutes						
	0	**10**	**20**	**30**	**40**	**50**	**60**
5%	3	5	5	2	3	3	3
10%	6	3	4	4	4	4	5
15%	5	9	5	4	2	3	3
20%	4	4	7	4	4	2	3

Table 5.8. Effect of the treatment duration (mins) × temperature (°C) interaction on percentage abnormal seedlings ($p<0.001$, $LSD_{0.05}$ 1.9).

	Duration minutes						
	0	**10**	**20**	**30**	**40**	**50**	**60**
45°C	5	5	5	3	5	5	5
50°C	4	6	6	6	5	4	5
55°C	4	5	5	2	1	1	0

From the analysis of the logistic curve μ (the 'average' time to germination) was significant ($p<0.001$) for temperature, duration and the temperature × duration interaction (Table 5.9), with the 55°C treatment showing a trebling of μ when the treatment duration increased from 0 (control) to 60 min.

Table 5.9. Effect of duration × temperature on μ ($p<0.001$, $LSD_{0.05}$ 0.70).

	Treatment duration (min)						
	0	**10**	**20**	**30**	**40**	**50**	**60**
45°C	3.8	3.7	3.9	4.2	3.9	3.9	3.7
50°C	4.2	3.7	4.0	4.2	4.0	3.9	4.2
55°C	4.4	3.8	4.9	5.8	7.1	8.6	11.8

Table 5.10. Effect of the treatment duration (mins) × temperature (°C) interaction on percentage dead seeds (p=0.002, $LSD_{0.05}$ 6.9) (left) and μ (p=0.022, $LSD_{0.05}$ 1.41) (right).

SMC (%)	Duration (mins)	Dead seeds (%) Temperature (°C)			μ Temperature (°C)		
		45	50	55	45	50	55
5	**0**	7	6	6	3.7	4.1	3.9
5	**10**	7	4	7	3.6	3.4	3.9
5	**20**	6	7	12	3.8	3.9	4.6
5	**30**	3	5	18	3.9	4.7	5.7
5	**40**	8	5	17	3.8	4.0	5.9
5	**50**	13	5	26	3.9	4.1	10.2
5	**60**	11	6	42	3.7	4.0	10.9
10	**0**	3	6	8	4.1	4.3	3.6
10	**10**	4	6	5	4.0	3.5	4.2
10	**20**	5	5	9	3.8	4.1	4.5
10	**30**	7	7	9	4.6	3.9	6.0
10	**40**	10	3	13	3.9	4.0	6.9
10	**50**	11	4	39	3.9	3.8	6.8
10	**60**	9	6	53	3.8	4.1	12.8
15	**0**	9	3	6	3.8	4.1	4.6
15	**10**	4	5	8	3.9	3.9	3.8
15	**20**	3	4	9	4.0	4.0	5.3
15	**30**	3	7	11	4.1	4.0	5.5
15	**40**	6	9	14	3.8	4.1	6.2
15	**50**	3	4	25	4.1	3.9	10.0
15	**60**	13	6	42	3.6	4.5	10.5
20	**0**	6	5	6	3.7	4.4	5.3
20	**10**	7	3	9	3.3	4.0	3.5
20	**20**	6	4	13	4.2	4.1	5.3
20	**30**	4	10	14	4.3	4.1	6.1
20	**40**	5	4	24	4.0	4.0	9.2
20	**50**	7	8	33	3.8	3.8	7.4
20	**60**	21	6	46	3.7	4.1	13.2

There was a significant (p=0.019) SMC × duration interaction on germination (Table 5.11) with a clear increase in μ with increasing duration but without a consistent change in μ from increased SMC. The three-way interaction of duration, temperature and SMC was also significant, with the majority of the effect being dominated by the effect of temperature (Table 5.10).

Table 5.11. Effect of SMC × duration on μ (in days) (p=0.019, $LSD_{0.05}$ 0.81).

	Treatment duration (min)						
	0	**10**	**20**	**30**	**40**	**50**	**60**
5%	3.9	3.6	4.1	4.8	4.6	6.1	6.2
10%	4.0	3.9	4.1	4.9	5.0	4.8	6.9
15%	4.2	3.8	4.5	4.5	4.7	6.0	6.2
20%	4.5	3.6	4.5	4.8	5.7	5.0	7.0

For γ (a measure of final germination), SMC (Table 5.12), temperature, duration, and the temperature × duration interaction (
Table 5.13) were all significant (p<0.001). Increasing SMC caused a decrease in γ, but the change was small. For temperature, duration and their interactions, at 55°C there was a significant and rapid decrease after 20 min, while it was not until 50 min that germination for the 50°C treatment declined. There was no change in germination at 40°C over time (

Table 5.13).

Table 5.12. Effect of SMC on γ (out of 50, p<0.001, $LSD_{0.05}$ 0.79).

	SMC %			
	5	**10**	**15**	**20**
γ	36.6	36.1	36.6	34.8

Table 5.13. Effect of duration × temperature on γ (p<0.001, $LSD_{0.05}$ 1.81).

	Treatment duration (min)						
	0	**10**	**20**	**30**	**40**	**50**	**60**
45°C	44.3	44.9	43.6	42.1	43.6	43.6	44.3
50°C	43.2	43.8	43.7	43.8	42.4	38.8	36.1
55°C	42.5	41.7	35.8	22.5	11.4	4.0	0.8

The temperature × SMC interaction was also significant (p=0.040) for γ (Table 5.14), but, it was dominated by the effect of temperature, as there was only a small decrease in γ with increasing SMC. The three way interaction was also significant (p=0.014) and was dominated by temperature and duration (Table 5.15).

Table 5.14. Effect of temperature × SMC on γ (p=0.040, $LSD_{0.05}$ 1.37).

	Seed Moisture Content			
	5%	**10%**	**15%**	**20%**
45°C	44.4	43.7	43.9	43.1
50°C	41.6	42.4	42.0	40.8
55°C	23.8	22.4	24.0	20.5

Table 5.15. Effect of the treatment duration (mins) × temperature (°C) × SMC (%) interaction on γ (p=0.014, $LSD_{0.05}$ 3.6).

SMC (%)	Duration (mins)	γ Temperature (°C) 45	50	55
5	**0**	45	43	43
5	**10**	45	43	43
5	**20**	43	43	38
5	**30**	44	45	24
5	**40**	44	41	16
5	**50**	45	39	2
5	**60**	44	38	2
10	**0**	43	46	43
10	**10**	44	45	42
10	**20**	44	44	36
10	**30**	42	43	23
10	**40**	45	42	10
10	**50**	43	38	4
10	**60**	45	38	0
15	**0**	45	43	43
15	**10**	46	46	41
15	**20**	43	44	37
15	**30**	43	43	25
15	**40**	42	44	6
15	**50**	44	39	1
15	**60**	44	36	14
20	**0**	44	42	41
20	**10**	44	42	41
20	**20**	44	43	33
20	**30**	40	44	18
20	**40**	44	43	3
20	**50**	42	39	0
20	**60**	44	32	6

Temperature (Table 5.16) and duration (Table 5.17) significantly affected β (the germination rate coefficient) ($p<0.001$). However, there was no clear trend for either of the parameters, which was especially visible for the factor duration with β initially increasing, then decreasing to 30 min and then increasing again. None of the interactions were significant ($p>0.4$).

Table 5.16. Effect of temperature on β (p<0.001, $LSD_{0.05}$ 0.075).

	Temperature °C 45	50	55
β	1.05	1.15	0.97

Table 5.17. Effect of treatment duration on β ($p<0.001$, $LSD_{0.05}$ 0.114).

	Treatment duration (min)						
	0	**10**	**20**	**30**	**40**	**50**	**60**
β	0.97	1.15	1.00	0.92	1.06	1.08	1.22

5.2.1.3. Discussion

The first experiment established the thermal treatment limits of carrot seed. Fifty-five degrees Celsius was clearly too hot, causing a significant decrease in germination after just 20 min. In comparison, germination started to decrease only after 40 min at 50°C, which suggests that 50°C can be considered the maximum safe temperature and 40 min the maximum duration. The results for dead seeds and abnormal seedlings support this, although they appear to be a less sensitive measure, as while the results for 55°C treatment show elevated percentage dead seeds and reduced abnormal seedlings increasing with treatment durations, at 50°C there is no change with duration. It is interesting that increased temperature and duration reduce abnormal seedlings. It may be that the defects that make the seedlings abnormal also make them more sensitive to negative conditions and more easily killed. This would appear to be in keeping with the results of seed vigour research (Hampton, 1999).

The germination rate analysis conducted in this experiment was in general agreement with the percentage germination data. However, γ (a measure of final germination) while following similar trends to percentage germination for treatment temperature and duration, demonstrated a slightly earlier onset of negative effects after 30 min at 50°C. This may indicate that damage to seeds is occurring more quickly than can be detected by simply assessing percentage germination. Seed vigour tests have shown that laboratory and field based tests determining percentage germination of heat treated seeds may not be in agreement with each other and that treatments found to be 'safe' under controlled condition tests may not be 'safe' when the seed is planted in the field. However, both measurements are measures of final germination, and the earlier decrease in γ was slightly less than the $LSD_{0.05}$, so the apparent earlier onset of seed damage it indicates should be treated with caution

While treatment temperature and duration had a large effect on germination for β (germination rate coefficient), there was no clear trend. The high level of significance without visible trends was likely to be due to the large statistical power of the experimental design. This is also partly true of SMC, where although the change in germination data is highly statistically significant, biologically the change is not sizeable, especially when compared to the large effects of temperature and duration on

germination. However, at the 5% SMC level there was a slight increase in the germination rate, indicating that lowering SMC before treatment could be beneficial in improving percentage germination.

5.2.2. Experiment 2: Effect of hot water treatment on infestation levels of *A. radicina* on carrot seed and carrot seed viability

5.2.2.1. Methods

Carrot seed infested with *A. radicina* was obtained from plants that had been inoculated by spraying laboratory-produced conidia onto them two months prior to harvest. A two factorial experimental design with SMC of 5, 10, 15 and 20% and duration of the hot water treatment of 0 (control) 10, 20, 30 min was conducted using the same methods used for Experiment 1 (section 5.2.1). In addition to measuring germination, percentage *A. radicina* infestation was determined. Ten seeds were placed on blotter paper that had been moistened with sterile distilled water in a 9 cm petri dish, incubated for three days in darkness at 20°C, then killed by placing in a -20°C freezer for 24 hours and incubated for 7 d at 20°C with alternating periods of 12 h near ultra violet light and darkness. Ten petri dishes (replicates) were completed for each treatment. Infestation was determined by visual identification of conidia on the seeds (ISTA, 2006). The methodology for measuring germination was the same as Experiment 1. Analysis was done by ANOVA on both untransformed and arcsine transformed data. No measurements of initial germination or *A. radicina* infestation were made.

5.2.2.2. Results

Data were normally distributed and as the transformation did not effect the results, untransformed data are presented.

SMC (Table 5.18) had a significant effect ($p=0.003$) on infestation with the tendency for decreasing levels of SMC to reduce infestation levels. Duration had a significant effect ($p<0.001$) on infestation levels with a very large reduction in infestation levels after ten minutes that was of biological significance (Table 5.19). The interaction of SMC × treatment duration was not significant ($p=0.347$).

Table 5.18. Effect of initial SMC on infestation levels of seeds by *A. radicina* ($p=0.005$, $LSD_{0.05}$ 4.0).

	SMC %			
	5	**10**	**15**	**20**
% infestation	18	23	20	25

Table 5.19. Effect of treatment duration on infestation levels of seeds by *A. radicina* ($p<0.001$, $LSD_{0.05}$ 4.0).

	Treatment duration (min)			
	0	**10**	**20**	**30**
% infestation	69	9	5	2

The grand mean for germination for the whole experiment was 16%, which was too low to allow for calculation and analysis of logistic curves of the germination data or to draw conclusions from.

5.2.2.3. Discussion

The second experiment showed that treatment duration had a very large and biologically substantial effect on the infestation levels of *A. radicina* reducing them to very low, agronomically acceptable, levels. The effect of SMC on infestation was less clear as there was not a clear trend in the data due to the infestation rate for 10% SMC being larger than that for 15% SMC. While the difference between the lowest and highest levels of SMC was biologically significant, these results require further qualification. The SMC of carrot seed is around 10% in ambient conditions, which indicates that no large reduction would be gained by reducing SMC to 5% in commercial operations. The high infestation levels and low overall germination of the infested seeds limits the ability to draw any clear conclusions from the germination analysis.

A methodological issue should be considered, in that the process of altering SMC may have a direct effect on the pathogen, as it does on the carrot seed. To be precise the experiment measures the effect of altering SMC on the pathogen as well, and to be completely thorough the effect of altering MC should be tested on the pathogen in isolation. However, the experiment is aimed at understanding and simulating a commercial practice so it is suggested this issue does not overly impinge the value of the study.

5.2.3. Conclusions

The safe treatment duration and temperature established for carrot seed is much greater that that required to cause a large reduction in viable *A. radicina*, showing that it is a practical and effective means of reducing infestation levels of carrot seed lots. The effect of altering SMC prior to treatment was less pronounced than in experiment 1 or than increasing treatment duration, a decrease in the infestation level. It may be commercially and practically valuable to decrease SMC to 5% prior to hot water treatment to reduce the loss of seed viability, while at the same time reducing viable fungal infestation levels. Even if reducing SMC is not commercially viable, care should

be taken to ensure that SMC is not raised above ambient levels prior to treatment, as this will reduce the effectiveness of the treatment.

These results are also in broad agreement with previous research, which found that 20 min at 55 °C or 30 min at 50°C give good control of *A. radicina* with minimal negative effects on the seed (Strandberg & White, 1989; Pryor *et al.*, 1994; Hermansen *et al.*, 1999). However, this and other research also shows that what was considered to be a safe and/or effective temperature × treatment duration combinations in one experiment was shown to be deleterious or less effective in another. This is reinforced by the variation among cultivars found by Strandberg & White (1989). The greater sensitivity to negative effects on seeds provided by analysis of germination rate further indicates that there may not be a single safe temperature × duration combination for all seed lots. Therefore, it would be prudent to test sub-samples of commercial lots of carrot seeds prior to treating the whole batch to determine the optimum treatment conditions for that particular seed lot.

Further work using seed with infestation levels more commonly found in commercial crops would be valuable to confirm the observed effects of altering SMC and also to test the method on other seed borne carrot pathogens such as *A. dauci* and *C. carotae*.

5.3. Agar compatibility tests for three carrot fungal pathogens and their potential biological control agents

5.3.1. Introduction

Four potential biological control agents (BCA) of *A. dauci*, *A. radicina* and *C. carotae* were identified in the literature review (section 2.3.3): *B. subtilis* (Serenade), *T. viride* (Trichoflow), *R. rhizogenes* (Dygall) and Effective Microorganisms (EM).

A laboratory-screening test of the BCA ability to control the pathogens was needed. The pathogens, which are mostly obligate on carrots, have been previously found to grow only, or at least better on, carrot leaf agar (CLA) than on standard fungal culturing agars such as potato dextrose agar (PDA) (Tuite, 1969; Strandberg, 1987; Carisse & Kushalappa, 1989; Strandberg, 2002). CLA is made by soaking 25 g of dried carrot leaves in one litre of distilled water for an hour, then straining the leaves from the resulting infusion, topping up with distilled water to 1 L and adding 15 g agar nobel. In comparison, PDA (Merck) contains 20 g glucose, 4 g potato extract and 15 g agar per litre, i.e. it contains more nutrients than CLA. Two of the BCA are bacteria, for which

the standard culture media is nutrient agar (NA) (Difco), which contains 3 g beef extract, 5 g peptone and 15 g agar per litre, which makes it quite different to both PDA and CLA. There was a need to find a growing media suitable for culturing both pathogens and BCA to enable valid screening tests.

Pre-experimental work plating EM found extensive variation in the constituent microorganisms that dominated, both within and between EM batches. Similar variation has been found by other researchers (van Vliet *et al.*, 2006). It was, therefore, considered inappropriate to include EM in any laboratory work, and that its testing should start with pot-based trials under controlled conditions in a glasshouse.

5.3.2. Experiment 1. Growth rates of *A. dauci*, *A. radicina*, *C. carotae* and *T. viride* on five agar formulations

5.3.2.1. Methods

Cultures of the three pathogens were purchased from the International Collection of Microorganisms from Plants (ICMP), Landcare Research New Zealand Ltd.: *A. radicina* ICMP10124, *A. dauci* ICMP10237 and *C. carotae* ICMP14651. *T. viride* was extracted from Trichoflow using serial dilutions by placing 0.1 g of Trichoflow in 9 ml sterile distilled water (SDW) shaking, and then aseptically withdrawing 1 ml and placing in a second container of 9 ml SDW and repeating to produce a dilution of 10^7. For the dilutions 10^5 to 10^7 0.1 ml was aseptically removed and spread on PDA agar plates and incubated until individual colonies were visible. Then five individual colonies from the 10^7 dilution plate were subcultured onto individual plates.

Five agars were used in this experiment: CLA as described above using 'Difco' agar nobel; PDA (Merck) 25 g L^{-1}; carrot leaf potato dextrose agar CLPDA using 25 g of PDA in place of 15 g agar nobel in CLA; NA (Difco) 23 g L^{-1} and carrot leaf nutrient agar (CLNA) where 23 g of NA replaces 15 g agar nobel in CLA. All agars were made up in 1 L of either distilled water or carrot leaf infusion and autoclaved for 15 min at 121°C.

Four plates of each agar were prepared for each pathogen and the BCA, each plate constituting one replicate. For *A. radicina*, *A. dauci* and *T. viride* plates were inoculated in the centre of the plate using a loop. For *C. carotae*, four evenly spaced inoculations were made on each plate. These were treated as sub-samples as they were considered to be pseudo-replicates as they were on the same plate, and they were averaged before analysis. Plates were individually sealed using a strip of low density polyethylene plastic wrap, their order randomised and placed in a growth cabinet at 20°C with a

12:12 light:dark cycle. *T. viride* colony diameters were measured daily, while *A. radicina* and *A. dauci* were measured every two days and *C. carotae* weekly. Colony diameter at the final measuring date was used in the analysis, as this showed the greatest variation between treatments and growth was found to be linear when plotted on a graph. *T. viride* was measured after 3 d, *A. dauci* 7 d, *A. radicina* 9 d, and *C. carotae* 47 d. The final measuring date for *T. viride* was three days, for *A. dauci* seven days, for *A. radicina* nine days and for *C. carotae* 47 d. Analysis was done by ANOVA using natural log transformed values with each species analysed separately.

5.3.2.2. Results

The growth rates, defined as the diameter of the fungal growth disk, of the three pathogens varied significantly among the five agars, but, there was no significant difference for *T. viride* (Table 5.20). The visual appearance of *A. dauci*, *A. radicina* and *T. viride* also varied among the agars with those containing carrot leaf infusion producing darker, greener hyphae compared to those without it (Figures 5.1. and 5.2 are examples of *A. dauci* growing on four different agar media).

Table 5.20. Mean colony diameter (mm) of three carrot fungal pathogens and one BCA on five agar formulations (log_e transformed data); *T. viride* was measured after 3 d, *A. dauci* 7 d, *A. radicina* 9 d, and *C. carotae* 47 d.

	CLA	CLNA	CLPDA	NA	PDA	$LSD_{0.05}$	p value
A. dauci	3.45	3.87	3.92	3.66	3.23	0.037	<0.001
A. radicina	3.51	3.59	3.62	3.99	3.28	0.025	<0.001
C. carotae	3.39	2.87	3.26	2.85	2.85	0.050	<0.001
T. viride	4.37	4.37	4.38	4.37	4.38	0.028	0.549

Figure 5.1. Growth of *A. dauci* on CLA (left) and CNA (right).

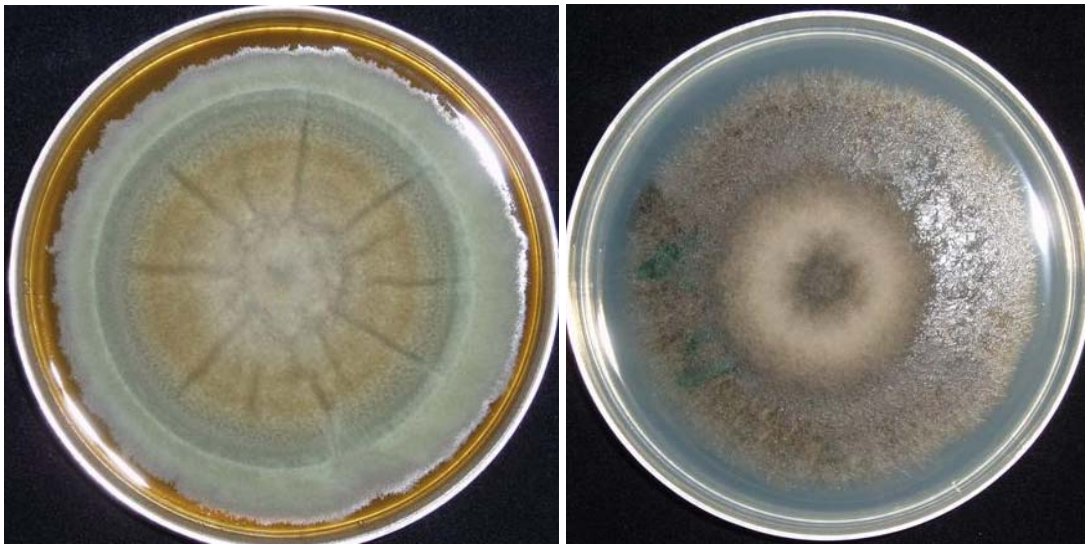

Figure 5.2. Growth of *A. dauci* on CPDA (left) and NA (right).

5.3.2.3. Discussion

While there was a significant difference between the growth of the three fungal pathogens and the fungal BCA on the different agars, it was not biologically large, indicating that the fungi grew successfully on all media. *T. viride* grew very fast, covering the plates within three days, compared to two weeks for *A. dauci* and *A. radicina*. *C. carotae* growth was very slow in comparison.

However, other authors have found that conidia production of the pathogens varies among agar formulations, including those tested here. This indicates that growth data alone can not be considered sufficient to determine agar suitability and that tests of conidia production are also required (Tuite, 1969; Strandberg, 1987; Carisse & Kushalappa, 1989; Strandberg, 2002). These tests are described in section 5.4.

5.3.3. Experiment 2: Growth of *B. subtilis* and *R. rhizogenes* on three agar formulations

5.3.3.1. Methods

The culture for *R. rhizogenes* came from the IMCP, number 3379, which is the strain used for Dygall. *B. subtilis* was extracted from Sentinel® using serial dilution the same as used for *T. viride* in section 5.3.2.1 except using NA plates. Both isolates were inoculated onto plates by a progression of streaks at right angles using a new disposable loop for each streak, to produce single cell cultures. Three agars were used (CLA, NA and CLNA) with four plates for each agar/BCA combination.

The normal incubation conditions for bacteria are 25°C in the dark. However, fungi are typically cultured at 20°C with 12 h light and 12 h in the dark. It was considered that the growth of the bacteria would be less affected under the standard fungal culture

conditions than vice versa. Thus, the culture conditions described for fungi in experiment one were also used for the bacteria. The growth of the bacteria cultures was visually assessed daily for five days.

5.3.3.2. Results

The growth of *B. subtilis* and *R. rhizogenes* varied among the three agars. *R. rhizogenes* produced pale cream coloured, smooth, shiny, raised circular cultures on NA, while the cultures on CLNA were darker and larger. The CLA cultures were the smallest and their circumference appeared less smooth compared to the cells growing on other media. *B. subtilis* also showed different morphologies on the three agars, forming roughly circular flattened cultures with indented edges and a pale cream colour on NA, while on CLA it produced similar looking cultures of a smaller size and with a light green/brown colour. On CLNA, cells were of much larger diameter and more irregular, with folds on the upper surface (Figure 5.3 shows examples of the growth of *R. rhizogenes* and *B. subtilis* on CLNA agar).

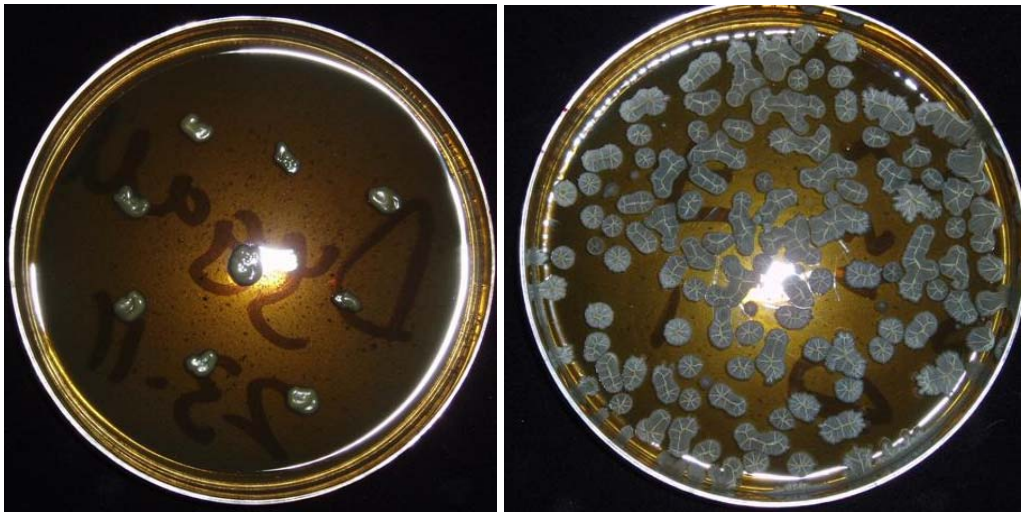

Figure 5.3. Growth of *R. rhizogenes* (left) and *B. subtilis* on CLNA.

5.3.3.3. Discussion

The two bacterial BCA grew successfully on all agars although there were notable differences in colony morphology.

While these tests are not considered definitive in determining the suitability of the agar formulations, there were no indications of negative effects that suggest an agar should not be used. All media were, therefore, included in subsequent tests.

5.4. Optimising conidia production by *A. dauci*, *A. radicina* and *C. carotae*

5.4.1. Introduction

Analysis of pathogens grown on agar has been shown in previous studies to be insufficient on its own to determine the suitability of an agar, and that measurement of conidia production is vital (Tuite, 1969; Strandberg, 1987; Carisse & Kushalappa, 1989; Strandberg, 2002). Further, if laboratory tests found that the BCA showed potential to control the pathogens, glasshouse trials on carrot plants would be necessary and this would require conidia to inoculate plants. Therefore, additional tests were conducted to study conidia production on different media. During these tests, it appeared that sporulation by *A. dauci* and *A. radicina* was partly in response to nutrient depletion as few conidia were produced until the fungi had covered the entire surface of the agar. The quantity of conidia produced by three inoculation methods were compared: a single central point on the agar plate made with a loop; ten evenly spaced points across the surface of the agar made with a loop; and water dispersion where liquid containing conidia and hyphal particles is spread across the surface of the agar using a 'hockey stick'.

5.4.2. Experiment 1: Conidia production of *A. dauci*, *A. radicina* and *C. carotae* on five agar formulations

5.4.2.1. Methods

Five agars were used (CLA, NA, CLNA, PDA and CLPDA) with four plates per agar and pathogen. *A. radicina* and *A. dauci* were inoculated in the centre of the plate using a disposable loop, while *C. carotae* was inoculated by using a disposable hockey stick to spread 100 µl of a solution of 0.01% Tween 80 in sterilised distilled water containing 3000 conidia of *C. carotae* ml^{-1}, adjusted using a haemocytometer. Plates were incubated under the same conditions as described in section 5.3.2 for seven days and then under 12 h near ultra violet light (NUV) light and 12 h dark for ten days at 20°C to promote conidia formation (ISTA, 2006).

Conidia were collected from the plates by putting 1.0 ml of a solution of 0.01% Tween 80 in sterilised distilled water onto each plate and gently rubbing the solution over the surface of the plate with a hockey stick and then draining it into a collection bottle. The collection bottle was hand shaken for three seconds then two 8 µl sub-samples were taken and placed in a haemocytometer to count the number of conidia.

The two sub-samples were averaged before analysis by ANOVA on untransformed data and square root transformed data. Each species was analysed separately.

5.4.2.2. Results

The data were normally distributed and the square root transformation did not alter the results, so only untransformed results are presented. There was a significant difference in the number of conidia produced on the different agars for *A. radicina* and *C. carotae* (Table 5.21). *A. dauci* data could not be analysed due to low counts but showed a similar trend to *A. radicina* and *C. carotae* (Table 5.21) with the highest numbers of conidia produced on CLA.

Table 5.21. Number of conidia μl^{-1} produced by *A. dauci*, *A. radicina* and *C. carotae* on five different agars.

	CLA	CLPDA	CLNA	PDA	NA	$LSD_{0.05}$	p value
A. dauci	71	40	13	0	5	n/a	n/a
A. radicina	914	113	109	69	18	96.7	<0.001
C. carotae	192	70	13	6	5	25.7	<0.001

5.4.2.3. Discussion

There were large biological as well as statistical differences among the different agars with CLA allowing the production of the largest number of conidia for all three species. The hypothesis that *A. radicina* and *A. dauci* produce conidia partly as a response to diminishing nutrient resources, which was the prompt for experiment 2, may also explain the lower conidia production on CLPDA, which contains more nutrients than CLA. If the plates had been incubated for longer, the position might be reversed and CLPDA produce more conidia. The higher numbers of conidia produced on CLA indicates that, while the pathogens grew satisfactorily on all agars tested, they were less or unsuitable for conidia production, which could mean that the growth displayed might be atypical.

5.4.3. Experiment 2: Effect of inoculation technique on *A. radicina* and *A. dauci* conidia production

5.4.3.1. Methods

A two factorial design with factors being agar type and inoculation method was used to determine the effect of inoculation technique on conidia production. The inoculation techniques were: single central point, ten evenly spaced points and water inoculation. Agar types were CLA, CLNA and CLPDA. For water inoculation a hockey stick was used to evenly spread 100 µl of a solution of 0.01% Tween 80 in sterilised distilled water containing 4000 conidia ml^{-1} (adjusted using a haemocytometer) across a plate.

Point inoculation was done by loop. Incubation, measurement and analysis were the same as described in experiment 1 except that incubation under UV light was reduced to five days from ten.

5.4.3.2. Results

The type of inoculation significantly affected conidia production in *A. radicina* (Table 5.22). Conidia numbers increased with increasing number of inoculation points on CLA but showed the opposite trend on CLPDA. The interaction was also significant ($p<0.001$) (

Table 5.23). *A. dauci* data could not be statistically analysed due to low conidia production, so only means are presented (Table 5.22).

Table 5.22. Number of conidia μl^{-1} produced by *A. radicina* and *A. dauci* on two agar types with three inoculation methods.

	CLA			CLPDA				
	Single	**Ten**	**Water**	**Single**	**Ten**	**Water**	**$LSD_{0.05}$**	**p value**
A. radicina	21	144	185	91	23	11	44.8	<0.001
A. dauci	10	8	5	0	0	0	n/a	n/a

Table 5.23. Number of conidia μl^{-1} produced by *A. radicina* on two agar types with three inoculation methods ($p<0.001$ $LSD_{0.05}$ 44.77).

	Single	**Ten**	**Water**
CLA	21	144	185
CPDA	91	23	11

5.4.3.3. Discussion

The larger number of inoculation points clearly increased conidia production by *A. radicina* on CLA but the opposite was found on CLPDA, which is contrary to expectations, especially if conidia production is in response to diminishing resources available to a colony, which should occur more quickly with more colonies per plate. Longer incubation periods may well be required to better study this effect. While the differences on CLA for *A. radicina* were considerable, the shorter incubation time was deliberately chosen to maximise the differences between the treatments, based on prior experience. While water inoculation proved useful if conidia were needed quickly, the additional complication of water inoculation over loop inoculation resulted in single or ten point inoculation being the preferred methods for conidia production.

The number of conidia produced by *A. dauci* on CLA for all inoculation methods were so small it is considered unwise to draw any conclusions. Further, the contradictory results for *A. dauci* between the two conidia tests for CLPDA can be explained by the raw data resulting from experiment 1 where only one replicate produced conidia and the others none.

Taking both experiments and previous results (Tuite, 1969; Strandberg, 1987; Carisse & Kushalappa, 1989; Strandberg, 2002) into account, CLA was considered the best media for growth and conidia production for all three pathogens. PDA and NA were considered unsuitable for culturing the pathogens or testing the BCA ability to control them. Due to the potential for atypical growth of the pathogens on agars other than CLA, it was concluded that subsequent biological control tests should be completed on both CLA and CLPDA for *T. viride* and CLA and CLNA for the bacteria. While the bacteria showed different colony morphologies on different agars, they all grew successfully and it was considered appropriate to use CLA and CLNA as culture and test media.

5.4.4. Conclusions

CLA is the preferred agar for culturing *A. dauci*, *A. radicina* and *C. carotae* and producing conidia. While loop inoculation of *A. radicina* and *A. dauci* produces conidia more slowly than water inoculation on CLA, it is simpler. As the BCA all grow satisfactorily on CLA, CLPDA and CLNA, all three media were used in the following tests to see if the BCA can control the pathogens.

5.5. The ability of four BCA to control carrot fungal pathogens *in vitro*

5.5.1. Introduction

This section studies the potential for the BCA to control *A. dauci*, *A. radicina* and *C. carotae in vitro* using the agar media developed in the previous section. Due to the different growth habits of the Alternaria fungi, *C. carotae* and the BCA, a range of different tests were required.

After completion of the literature review and initial laboratory tests, a new BCA was released in New Zealand under the trade name Sentinel to control *Botrytis cinerea* on grape vines. The constituent microorganism, *Trichoderma atroviride* strain LC53, is closely related to *T. viride*, which is the active component in Trichoflow (Stewart *et al.*, 2005). Sentinel® has been formulated for foliar application compared to the soil and hydroponic application of Trichoflow. This, coupled with its ability to control *B. cinerea*, meant it was considered worthwhile to include it in the testing regime. Agar based tests were conducted to determine the ability of *T. atroviride* to control the pathogens but not BCA compatibility tests.

5.5.2. Experiment 1. Control of *A. dauci* and *A. radicina by R. rhizogenes* and *B. subtilis*

5.5.2.1. Methods

The interaction of *A. dauci* and *A. radicina* with *R. rhizogenes* and *B. subtilis* was studied by inoculating the centre of an agar plate with the pathogen using a loop. After two days of incubation at 20°C under a 12 h dark/12h light regime, the plates were inoculated with the BCA in a square pattern, with each of the four colonies being approximately one third of the way from the edge to the centre of the plate. For each agar/pathogen/BCA combination six plates were used, three of which were not inoculated with the BCA as untreated controls. Plates were individually wrapped and their order within the incubator was randomised. Plates continued to be incubated under the same conditions for up to two weeks. Plates were visually inspected for zones of inhibition between the pathogen and BCA.

5.5.2.2. Results

B. subtilis showed strong control of *A. dauci* confining the pathogen to the centre of the plate on CLA and CLNA (Figure 5.4). *B. subtilis* slightly slowed the growth rate of *A. radicina* compared with the controls and there were slight zones of inhibition formed on CLNA. Despite the slower growth, *A. radicina* overgrew the *B. subtilis* colonies. *A. radicina* had no inhibition zones around *R. rhizogenes* colonies and overgrew the bacterial colonies with little impediment on CLA, but grew more slowly on CLNA (Figure 5.4). *A. dauci* also showed no inhibition zones and overgrew *R. rhizogenes* colonies on both agars but more slowly on CLNA.

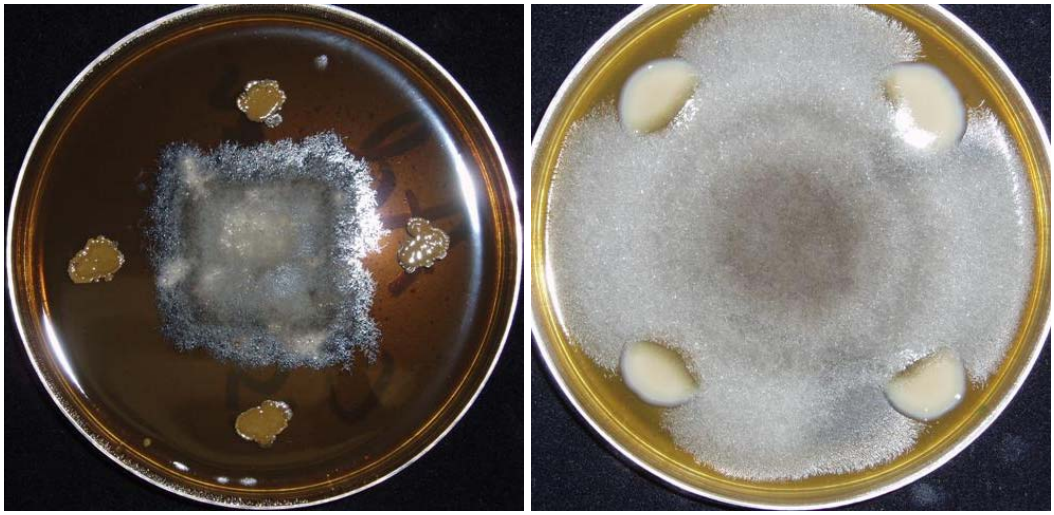

Figure 5.4. Suppression of *A. dauci* by *B. subtilis* on CLA (left) and limited suppression of *A. radicina* by *R. rhizogenes* on CLNA (right).

5.5.3. Experiment 2. Control of *C. carotae* by *R. rhizogenes* and *B. subtilis*

5.5.3.1. Methods

C. carotae grows much more slowly than the other pathogens, reaching a diameter of only 3 cm after fifty days, so the approach used in the first two experiments could not be used for this pathogen. Instead, *C. carotae* was streaked across the centre of a plate and after either two or five days of incubation one streak of *R. rhizogenes* or *B. subtilis* was made at a ninety-degree angle to the *C. carotae* streaks, forming a cross. CLA and CLNA agars were used with six replicates (plates) for each incubation period/BCA/agar combination, three of which were untreated controls (no BCA). Plates were individually wrapped and the position within the incubator was randomised. Incubation conditions were the same as for experiment one but plates were incubated for three weeks. Plates were visually inspected for zones of inhibition between pathogen and BCA.

5.5.3.2. Results

When *B. subtilis* was streaked across the plate two days after *C. carotae* inoculation, there was clear suppression of *C. carotae* colonies nearest the streak, with both the population and size of *C. carotae* colonies increasing with greater distance from the streak (Figure 5.5). When *B. subtilis* was streaked five days after *C. carotae* inoculation, the suppression level was markedly reduced with only a slight reduction in *C. carotae* colony size and population next to the *B. subtilis* streak compared to the plate edge (Figure 5.5). The results were the same on both agars. *R. rhizogenes* had no effect on *C. carotae* colony population or size on either agar formulations (Figure 5.6).

Figure 5.5. Suppression of *C. carotae* by *B. subtilis* streaked onto plate 2 d after *C. carotae* inoculation (left), limited suppression of *C. carotae* by *B. subtilis* streaked onto plate 5 d after *C. carotae* inoculation.

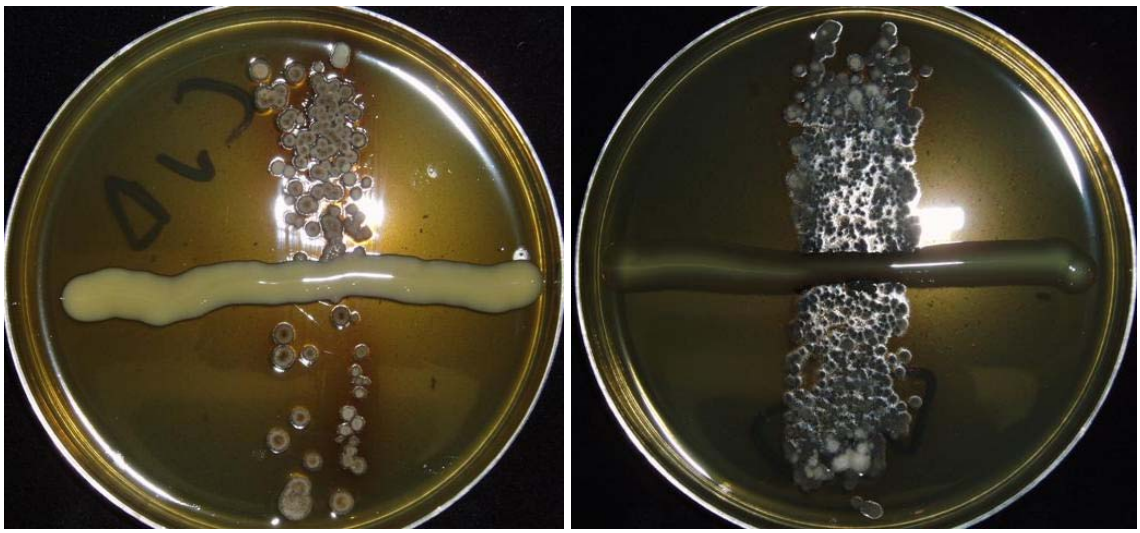

Figure 5.6. No suppression of *C. carotae* by *R. rhizogenes* streaked onto plate 2 d after *C. carotae* inoculation CNA (left) CLA (right).

5.5.4. Experiment 3. Control of *A. dauci, A. radicina* and *C. carotae by T. viride* and *T. atroviride*

5.5.4.1. Methods

The interactions of *A. dauci*, *A. radicina* and *C. carotae* with *T. viride* and *T. atroviride* were determined by comparing the growth of the pathogens in the presence and absence of the BCA on CLA and CLPDA. *A. dauci* and *A. radicina* were inoculated at the centre of a plate using a loop and grown for six days before inoculum of *T. viride* or *T. atroviride* was streaked around the outside edge of the agar plate with a loop. In contrast, *C. carotae* was inoculated by loop at four equidistant points and grown for 14 d before inoculating with *T. viride*, and for 22 d and 32 d for *T. atroviride* on CLPDA and CLA, respectively. *T. viride* and *T. atroviride* were inoculated at the centre of the plate using a loop. Four replicates (plates) were used for both treated and untreated controls. Plates were individually wrapped and the position in the incubator was randomised. Pathogen colony diameter was measured five times over 44 d for *C. carotae* and five times over 22 d for *A. radicina* and *A. dauci*. The four colonies per plate for *C. carotae* were treated as sub-samples and averaged before analysis. Growth curves were drawn and a two factorial (BCA and agar) ANOVA was performed on untransformed and log-transformed data for the final measurements as these showed the greatest variation between the treatment and control.

5.5.4.2. Results

As data were normally distributed and transformation did not alter the results, only untransformed data are presented. Both *T. viride* and *T. atroviride* caused a complete cessation of the growth of *A. dauci*, *A. radicina* and *C. carotae* on all agars (Examples

are given in Figures 5.7, 5.8 and 5.9). There was a highly significant difference between the treated plates and the untreated controls for all BCA and pathogen combinations (Tables 5.24 & 5.25).

Table 5.24. Colony diameter (mm) of *A. dauci*, *A. radicina* and *C. carotae* in the presence or absence of *T. atroviride* on CLA and CLPDA. *C. carotae* measured after 44 d *A. dauci* and *A. radicina* after 22 d.

	CLA		CLPDA			
	T. atroviride	**Control**	***T. atroviride***	**Control**	**$LSD_{0.05}$**	**p value**
A. radicina	36	82	36	73	10.0	<0.001
A. dauci	45	82	49	82	3.9	<0.001
C. carotae	18	22	14	22	3.7	<0.001

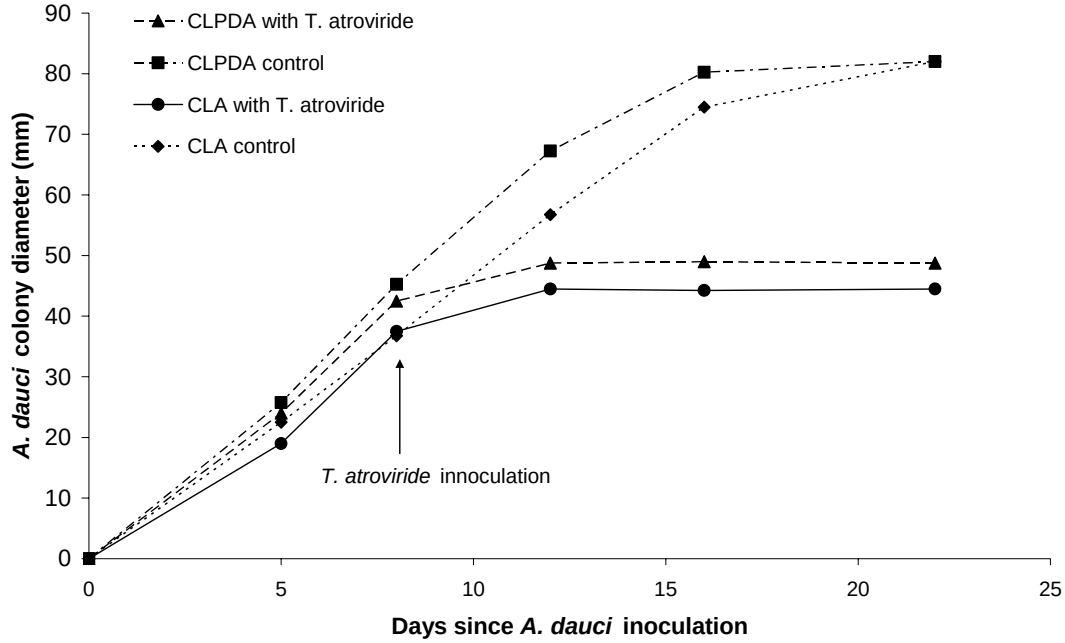

Figure 5.7. Growth of *A. dauci* in the presence or absence of *T. atroviride* on CLA and CLPDA.

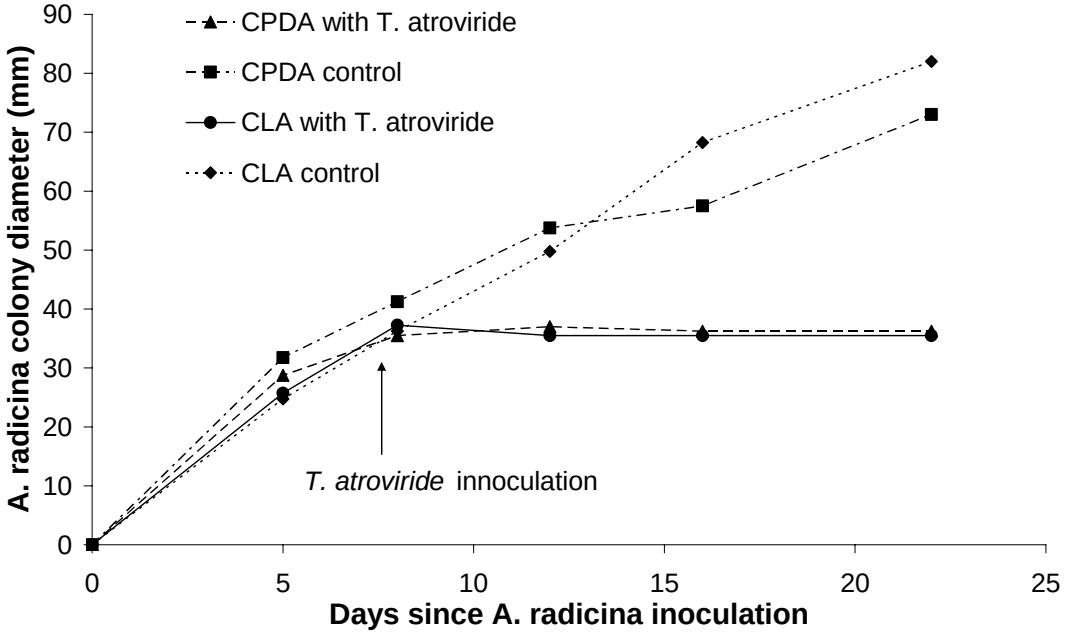

Figure 5.8. Growth of *A. radicina* in the presence or absence of *T. atroviride* on CLA and CLPDA.

Table 5.25. Colony diameter (mm) of *A. dauci*, *A. radicina* and *C. carotae* in the presence or absence of *T. viride* on CLA and CLPDA. *C. carotae* measured after 44 d *A. dauci* and *A. radicina* after 22 d.

	CLA		CLPDA			
	T. viride	**Control**	***T. viride***	**Control**	**$LSD_{0.05}$**	**p value**
A. radicina	35	82	36	67	11.3	<0.001
A. dauci	52	82	54	82	1.9	<0.001
C. carotae	10	20	11	23	1.2	<0.001

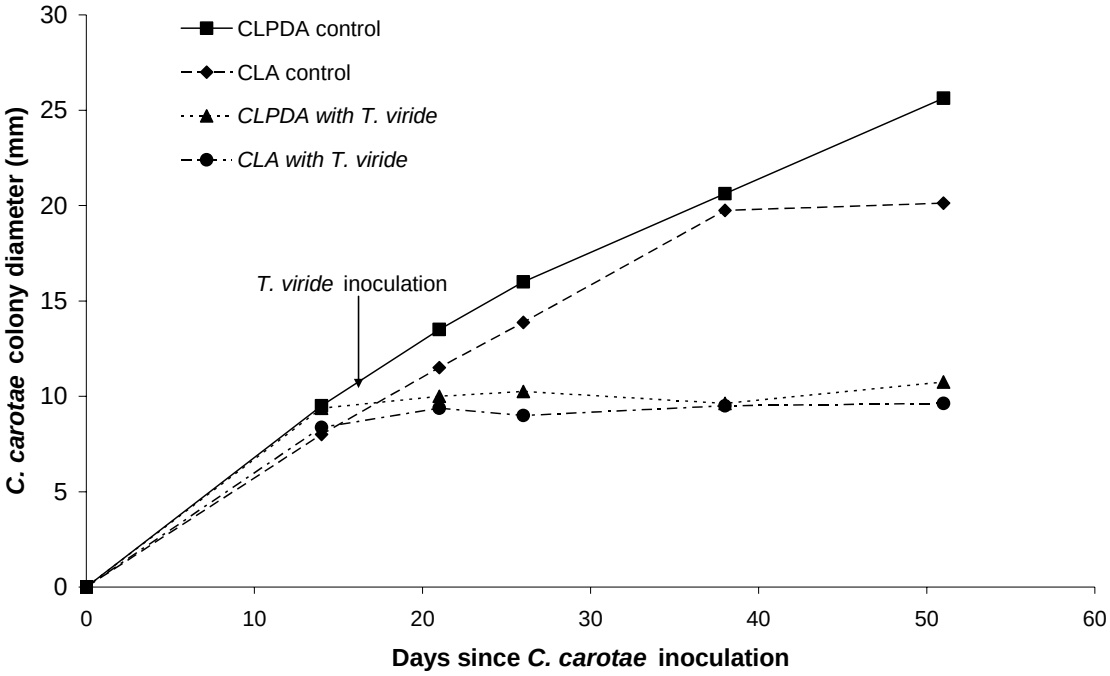

Figure 5.9. Growth of *C. carotae* in the presence or absence of *T. viride* on CLA and CLPDA.

5.5.5. Discussion

R. rhizogenes showed no significant control over any of the pathogens under controlled conditions. It is therefore considered unlikely that this organism will achieve control of any of the pathogens under field conditions. *B. subtilis*, on the other hand, showed good inhibition of *A. dauci* but limited effect against *A. radicina*, and was only effective against *C. carotae* shortly after inoculation with the pathogen. Therefore, *B. subtilis* may have some potential for use against fungal pathogens on carrots in the field, especially as it is formulated for agricultural use and is known to be effective against a range of fungal pathogens (Walton, 2001; O'Connor, 2002). *T. viride* and *T. atroviride* showed strong control of all three pathogens causing a complete halt of their growth. This, coupled with their existing use as bio-fungicides, indicates that they may have a potential to control the carrot pathogens in the field.

The lack of control of *A. radicina* by *B. subtilis* is dissimilar to the results of Catska (1989), who found that the bacterium strongly controlled *A. alternata in vitro* as well as *Penicillium claviforme* Bainier, *Penicillium expansum*, *Penicillium griseofulvum* Dierckx., *Gaeumannomyces graminis* (Sacc.) Arx and D. Olivier and *Cercosporella*

herpotrichoides Fron. There are increasing numbers of *A. radicina* strains being identified (Farrar *et al.*, 2004), so further work using a range of both *A. radicina* and *B. subtilis* isolates is necessary to see if the effectiveness of the BCA varies for different isolates.

5.5.6. Conclusions

It was concluded that *T. viride*, *T. atroviride* and *B. subtilis* should be tested for their biocontrol potential of fungal pathogens on carrots in glasshouse trials

5.6. Self compatibility of biological control agents

5.6.1. Introduction

This section examined the compatibility of the BCA with each other to see if there is potential for them to be used in combination, as is done with synthetic fungicides (Walton, 2001; O'Connor, 2002), in order to gain better control of the pathogens than one BCA could achieve on its own. This work was completed before *T. atroviride* was introduced, so this fungus was not included in these tests.

5.6.2. Experiment 1. Compatibility of *B. subtilis* and *R. rhizogenes*

5.6.2.1. Methods

The compatibility of *B. subtilis* and *R. rhizogenes* was determined by taking a sample from a single cell colony of *B. subtilis* and making a spot inoculation on a NA plate using a loop. A new loop was used to take a sample from a single cell colony of *R. rhizogenes* and inoculate the same spot on the agar. The organism inoculated first was alternated, two inoculations were made on each of four plates and the inverted plates were incubated at 25°C in the dark for two days. A sample was then taken from each colony, mixed with 9 ml of sterile distilled water and a dilution series was prepared to a dilution of $1:10^6$. Spread plates were prepared with 100 µl of the $1:10^4$ to $1:10^6$ dilutions on CLNA using the spread plate method (Staley, 1996). CLNA was used due to the distinctive folded morphology *B. subtilis* exhibited on it. The cultures were incubated under the same conditions for a further three days and visually inspected for cultures of both organisms.

5.6.2.2. Results and Discussion

No colonies of *R. rhizogenes*; were found, however, *B. subtilis* colonies were established on plates of all dilutions.

B. subtilis and *R. rhizogenes* were clearly incompatible under the experimental conditions, with *B. subtilis* eliminating, or inhibiting the growth of *R. rhizogenes* meaning that it is unlikely that the two BCA will be compatible under field conditions, except perhaps for short durations.

5.6.3. Experiment 2. Compatibility of *T. viride* with *R. rhizogenes* and *B. subtilis*

5.6.3.1. Methods

The compatibility of *T. viride* with *R. rhizogenes* and *B. subtilis* was tested using the same methodology as described in section 5.5.2, except that *R. rhizogenes* and *B. subtilis* were inoculated onto the plate two days before *T. viride* and that CLA, CLPDA and CLNA agars were used.

5.6.3.2. Results and discussion

T. viride completely overgrew *R. rhizogenes* on all three agars, while clear zones of inhibition existed between *T. viride* and *B. subtilis* on all agars (examples are shown in Figure 5.10). However, on CLNA, *T. viride* overgrew some *B. subtilis* colonies after ten days. The lack of zones of inhibition between *T. viride* and *R. rhizogenes* is an initial indication that these two species may be compatible with each other. It would need to be established further if *R. rhizogenes* will grow in the presence of *T. viride* and *T. atroviride*, especially when combined in agricultural spraying equipment and applied to crop foliage. However, due to the lack of pathogen suppression by *R. rhizogenes* no further compatibility tests were undertaken, as this BCA was not going to be tested in glasshouse trials. *T. viride* and *B. subtilis* were clearly incompatible when grown together on an agar plate for two days. However, shorter term mixing, e.g., within an agricultural spray applicator, may be possible.

5.6.4. Conclusions

B. subtilis and *T. viride*, the BCA which showed the greatest potential to control the pathogens *in vitro*, also showed the strongest antagonism to each other, indicating a lower likelihood of being compatible if mixed in the same spray formulation and/or coexisting on plant foliage. If *B. subtilis, T. viride* and/or *T. atroviride* show potential in glasshouse or field trials, then more detailed *in vivo* testing of the compatibility of the three BCA should be undertaken.

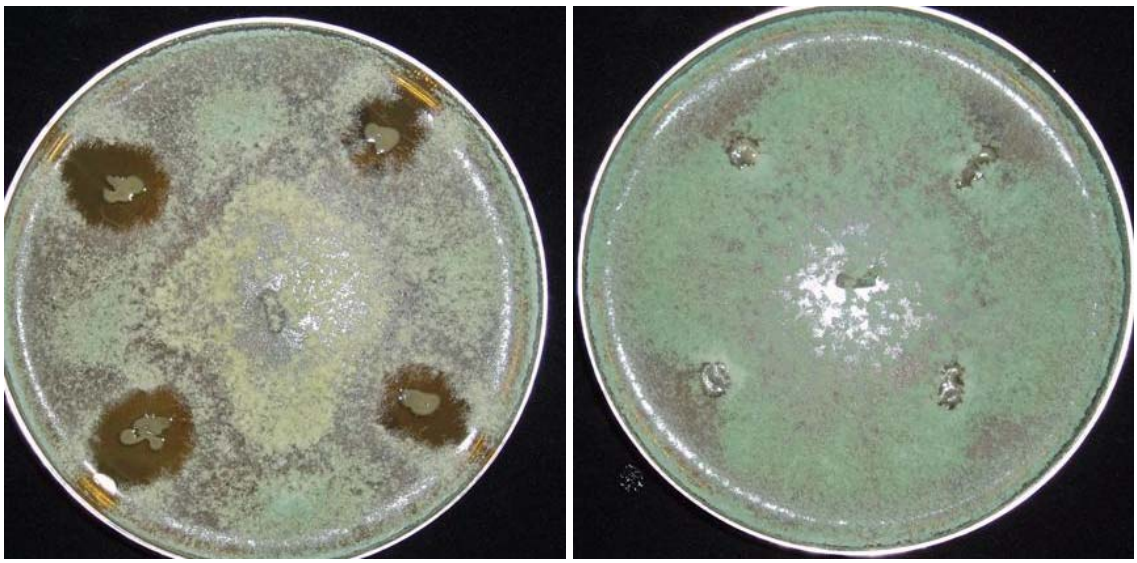

Figure 5.10. Zones of inhibition between *T. viride* and *B. subtilis* on CLA (left), no inhibition of *T. viride* by *R. rhizogenes* on CLA (right).

5.7. The control of carrot fungal pathogens by BCA under controlled conditions

5.7.1. Methods

A series of randomised complete block design experiments was used to test the ability of *B. subtilis*, *T. atroviride*, *T. viride* and Effective Microorganisms (EM) to control *A. dauci*, *A. radicina* and *C. carotae*. For each pathogen, two BCA application times were tested: applying the BCA 24 h before applying the pathogen and 24 h after applying the pathogen. Each application time for each BCA constituted a separate trial.

EM is usually supplied as a stock solution from the manufacturer and 'multiplied' on-farm by mixing 1 L of stock solution and 1 L of molasses in 18 L of water, and maintaining the mixture at a constant temperature of 30°C for seven days. For these trials, EM stock solution was supplied by the New Zealand manufacturer, the New Zealand Nature Farming Society Inc. (Christchurch, New Zealand) and was diluted at 1 L EM in 9 L water. The dilution rates for the other BCA were: 90 g of Serenade, 3 g Trichoflow and 4 g Sentinel, respectively, dissolved in 10 L of water. Solutions were shaken immediately to mix thoroughly and sub-samples of the required volume were taken and used within one hour.

Pathogen conidia to inoculate the carrots were produced according to the methods described in section 5.4 using a single, central point inoculation on CLA. The number of conidia were adjusted to 5,000 conidia ml^{-1}. Carrot plants were inoculated by spraying each conidia suspension through an airbrush onto the plants at a rate of 0.5 ml

per plant. BCA were applied to the plants using the same technique except 1 ml per plant was applied.

Carrots were grown in plant raising trays (60 cells per tray; cell volume of 42 cm^3) with one carrot plant per cell in an unheated glasshouse until foliage was approximately 8 cm high. Each separate experiment comprised one pathogen, one pathogen application time (application before or after the BCA) and four BCA. Each block contained four carrots treated with both BCA and pathogen, four carrots treated only with the BCA (BCA control), and four carrots treated only with the pathogen (pathogen control). There were four blocks resulting in 48 plants per trial.

For the experiments where the BCA was applied first, the plants were returned to the glasshouse after BCA application for 24 h. The plants were set out in the glasshouse in the randomised complete block (RCB) layout using two plant raising trays such that the foliage of different plants did not touch, to reduce the potential for cross contamination. After 24 h, the pathogen was applied to the carrots *en masse* (except for the BCA controls). The trays were placed into plastic containers with clear lids containing approximately 2 cm of water, and maintained at 25°C with 12 h dark and 12 h fluorescent light for 24 h to produce favourable infection conditions for the pathogens (Lauritzen, 1926; Strandberg, 1987; Carisse & Kushalappa, 1989, 1992; Carisse *et al.*, 1993). The trays were then removed from the containers, returned to the glasshouse and kept for 9 d. Disease levels were recorded on days 3, 6 and 9. For the experiments where the BCA was applied after the pathogen, carrot plants were inoculated with the pathogens *en masse* (with the exception of the BCA control) and all plants, including the BCA controls, were immediately placed in the plastic containers with clear lids under inoculation conditions for 24 h. The plants were then removed from the containers, the BCA was applied to all plants except the pathogen controls, the plants were arranged in the trays in the randomised complete block layout and placed in the glasshouse for 9 d. Disease severity was assessed using the 'disease damage index' of Strandberg (1988) on two older leaves from each carrot plant to give a percent necrotic tissue (values from the two leaves were averaged before analysis). For all the trials, disease severity was greatest at day nine, so only those values were used for analysis by ANOVA on untransformed and ARCSINE transformed data.

5.7.2. Results

All data were normally distributed and as ARCSIN transformation did not alter the results, only untransformed data are presented. The BCA controls were disease free and

thus were excluded from the analysis. There were no significant results except for *A. dauci,* where EM was applied first which resulted in an increase in fungal infestation (Table 5.26). The biological differences between BCA treatments and the pathogen control were very small, even when considering the statistically significant *A. dauci* and EM result.

Table 5.26. The effect of four BCA on the percentage area infection of *A. dauci*, *A. radicina* and *C. carotae* on young carrot leaf foliage. BS = *B. subtilis*, EM = Effective Microorganisms, PC = pathogen control (only pathogen applied), TA = *T. atroviride*, TV = *T. viride*. Applied indicates if the pathogen was applied before the BCA or after the BCA.

Pathogen	Applied	PC	BS	EM	TA	TV	$LSD_{0.05}$	p value
A. dauci	**Before**	17	17	22	18	18	2.4	0.002
	After	19	17	20	18	19	2.7	0.517
A. radicina	**Before**	24	26	26	25	25	4.7	0.840
	After	25	26	27	26	25	2.7	0.622
C. carotae	**Before**	17	15	17	16	15	3.8	0.736
	After	15	18	16	16	16	5.4	0.917

5.7.3. Discussion

There was no evidence of the BCA controlling the pathogens. The one statistically significant result conversely found that the BCA increased infestations levels. However, as the biological effect was small this may be a chance result.

After the BCA application to the plants they were returned to the glasshouse, rather than the plastic containers as for the pathogens. It is possible that placing the plants in the containers may have given the BCA more optimal conditions allowing them to better control the pathogens. This was not done as it was considered that returning the plants to the glasshouse would be closer to field conditions and optimal conditions for the BCA had not been established. An alternative approach would be to not use the high humidity containers and allow pathogen infestation to occur under glasshouse conditions. This could be a more realistic test, but, previous research has found that under such conditions infestation levels are low, so that greater numbers of plants and/or replicates may have been needed to establish an effect.

The lack of disease control is disappointing as *in vitro* results indicated that some level of control could be expected. However, the conditions in the trial, while carefully controlled, were optimised for the pathogens, using large numbers of conidia to inoculate plants as well as providing favourable infestation conditions. In the field there will most likely be lower numbers of conidia present on the leaves, and temperature and relative humidity (RH) may be sub-optimal for pathogen growth and infestation. Therefore, the performance of the pathogens under field conditions may differ from the

results presented here. However, the BCA are also living organisms so they may also be negatively affected by field conditions and those of this trial. It is, hence, difficult to extrapolate the results of the glasshouse trial to performance in the field. Therefore, it was decided to complete a field trial testing the ability of the BCA to control *A. radicina* and *A. dauci*.

5.8. The control of *A. radicina* and *A. dauci* by BCA under field conditions

5.8.1. Methods

Two separate, randomised complete block trials were conducted, one irrigated on the soil surface by drip tape laid on top of the soil and the second irrigated over the top of the crop by sprinklers. The trials were conducted at the Biological Husbandry Unit, Lincoln University, New Zealand (43°39'005''S-172°27'250''E), on a Wakanui silt loam from August 2005 to March 2006. There were five treatments, *B. subtilis*, *T. viride*, *T. atroviride* and EM and an untreated control, with five blocks per trial. The formulations were the same as used in the pot based disease trial described in section 5.6. To ensure its efficacy on crop foliage, *T. atroviride* had to be tank-mixed at 0.5 ml L^{-1} with the sticker/spreader/UV protectant NuFilm-17 (Miller Chemical and Fertiliser Corporation, Pennsylvania, USA). As *T. atroviride* and *T. viride* are both soil inhabiting fungi, and *T. viride* is formulated for use in soil and hydroponic systems NuFilm-17 was also mixed with the *T. viride* spay solution at 0.5 ml L^{-1} to assist it to persist on plant foliage. All treatments were applied at a rate of 1.3 L m^{-2} at 1 bar through a custom-built sprayer using XR TeeJet 110 04 UP nozzles (Spraying Systems Co. Wheaton, Illinois, USA), spaced 50 cm apart along the spray boom and positioned approximately 50 cm above the crop. This was considered a high application rate, which wetted the crop to run-off. Spraying started in mid December and was applied approximately weekly depending on weather conditions, which was considered an intensive spraying regime. Spraying was completed in the early morning under still conditions to minimise spray drift and was postponed if it was raining or rain was forecast for later that day to minimise the likelihood of the BCA being washed off the plants.

The F1 hybrid parent lines of the carrots planted were A680059 (seed parent) and C680069 (pollen parent). A680056 is considered to be highly susceptible to carrot fungal pathogens, especially to *A. radicina* (James Smith pers. comm. 2005). The seed parent plots were 1×3 m with four rows spaced 33 cm apart with carrots planted every

10 cm in the row to give a target density of 40 plants m^{-1}. The steckling carrots were obtained from a commercial grower and planted on 20th August 2005. Between each seed parent plot in each block and at the ends of each block was a single 3 m long row of the pollen parent cultivar. There was a 1.5 m gap between the seed parent plot and pollen parent row and a 2 m gap between blocks to provide a spray drift buffer between seed parent plots. The inter-block gap was sown with grass to facilitate tractor access, while the plots and inter-plot gap had bare soil, which was kept weed-free mechanically. There were two lines of drip tape for each female plot and one for each male row in the surface irrigated experiment, while in the overhead-irrigated trial 16 sprinklers were spaced so that they evenly wetted the whole trial area. Irrigation was applied as for commercial crops: when soil was dry to 5 cm depth, the soil was brought up to field capacity. In addition, the overhead-irrigated trial was also given approximately 15 min watering in the afternoon/evening from the 3rd February until 10th March, if weather conditions were dry, with the aim of increasing disease pressure.

Approximately fortnightly, from 21 December 2005 to 22 March 2006, a visual assessment of the disease level of each plot was made based on a disease index created for this trial (Table 5.27).

Table 5.27. Disease index for carrots, with disease taken to be areas of stem and leaves showing browning and/or necrotic areas.

Index value	Description of disease level
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 stem diseased
10	Leaves senesced and all stems diseased

The seed parent cultivar was harvested on 25 March and the whole umbels were left to dry indoors for a week. The umbels were threshed in a Wintersteiger LD 350 thresher (Wintersteiger AG., Dimmelstrasse, Austria) using a 2.6 × 7.5 mm screen. The seed was graded using a Westrup seed cleaner (Westrup A/S, Slagelse, Denmark, model LA-LST): the scalping screen was 3.0 mm round, the top screen 1.75 mm slotted and bottom screen 0.7 mm slotted with a shaker motor speed of 400 rpm. The fan settings

were pre-aspiration gate 1, filter valve 1.5, fan outlet valve 10. The seed was then passed through the indented drum section of the cleaner using a 3.0 mm indent.

The yield of cleaned seed from each plot was weighed and sub-samples were obtained using a rotary seed divider (Hoffman Manufacturing Co., Oregon, USA, model HMCUD). The seed was tested for germination following the method described by ISTA (2006) except that rolled towels were used in place of blotters. Seed was tested for the presence of *A. dauci* and *A. radicina* following the method described by ISTA (2006). However, there were large populations of a range of other fungal species on the seed, which appeared to be suppressing *A. dauci* and *A. radicina* colonies. The seed was therefore retested after surface sterilising it in 1.0% sodium hypochlorite solution for 5 min. In addition, the ten Petri dishes described in the ISTA (2006) tests for *A. dauci* and *A. radicina* were substituted by a single plastic accelerated aging box (11.0 × 11.0 × 3.5 cm) (ISTA, 2006) lined with three blotters to reduce handling time.

Crop debris was also tested for the presence of *A. dauci* and *A. radicina*. For this, eight 8 cm long pieces of crop stems between 5 to 10 mm in diameter were collected from each plot. They were surface sterilised in 1.0% sodium hypochlorite solution for 5 min, then incubated at 25°C under 12 h NUV/12 h dark for 7 d in accelerated aging boxes containing three moistened blotters. They were then visually inspected for *A. dauci* and *A. radicina* as per the methods for the detection of *A. radicina* and *A. dauci* on seed (ISTA, 2006) .

Results were analysed by ANOVA on untransformed and log-transformed data for weight measurements and ARCSINE transformed data for percentage measurements. For the disease index measurement, the GenStat V8.2.0 'POL' function was used to fit linear, quadratic and cubic curves to disease index curves to produces a p value for the fit of each curve to the data.

5.8.2. Results

Data were normally distributed and transformations did not alter the results, so only untransformed data are presented, except for crop debris and *A. dauci* infestation of seeds were there were too few data to produce a normal distribution even after transformation. In this case, only means are presented.

Visual disease index levels increased throughout the measurement period (Figures 5.11 & 5.12). There was a significant fit ($p<0.001$) for linear, quadratic and cubic curves for the disease index in both trials, except for the cubic curve for the trickle

irrigated trial (p=0.266), indicating that the response curve is complex for the sprinkler irrigated trial and non-linear for the drip-tape trial (Figures 5.11 & 5.12). The comparison of density effect curves for each treatment did not reveal any significant differences between curves (p>0.3 for all comparisons) indicating that the curves were the same for all treatments in both trials.

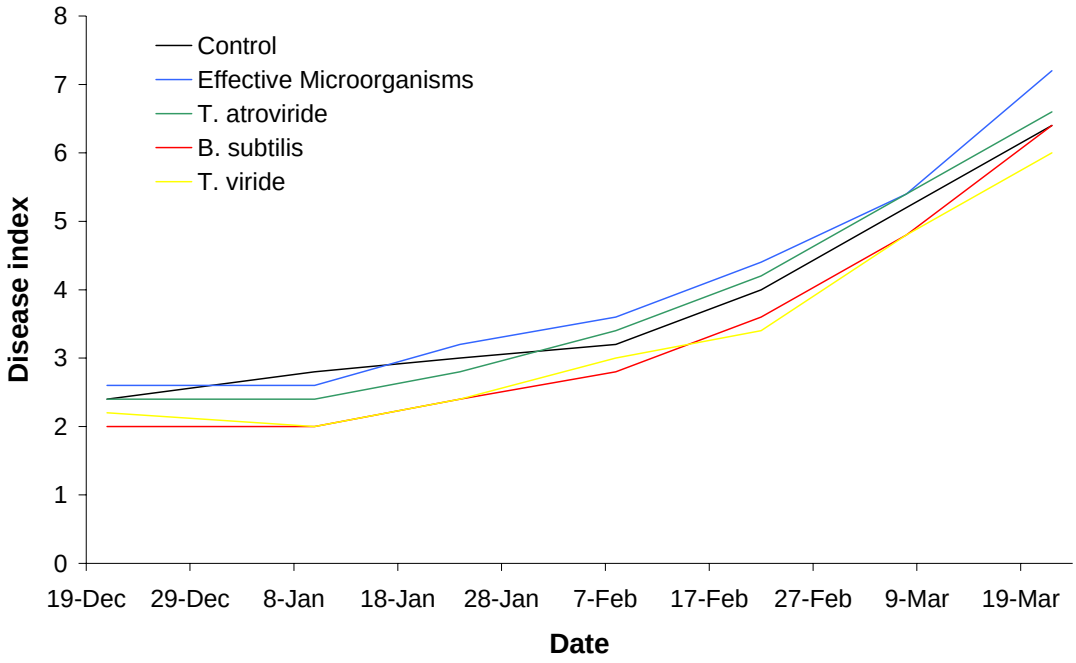

Figure 5.11. Disease symptoms on carrot plants in surface irrigated trial based on a disease index ($LSD_{0.05}$ 0.24 for treatment means).

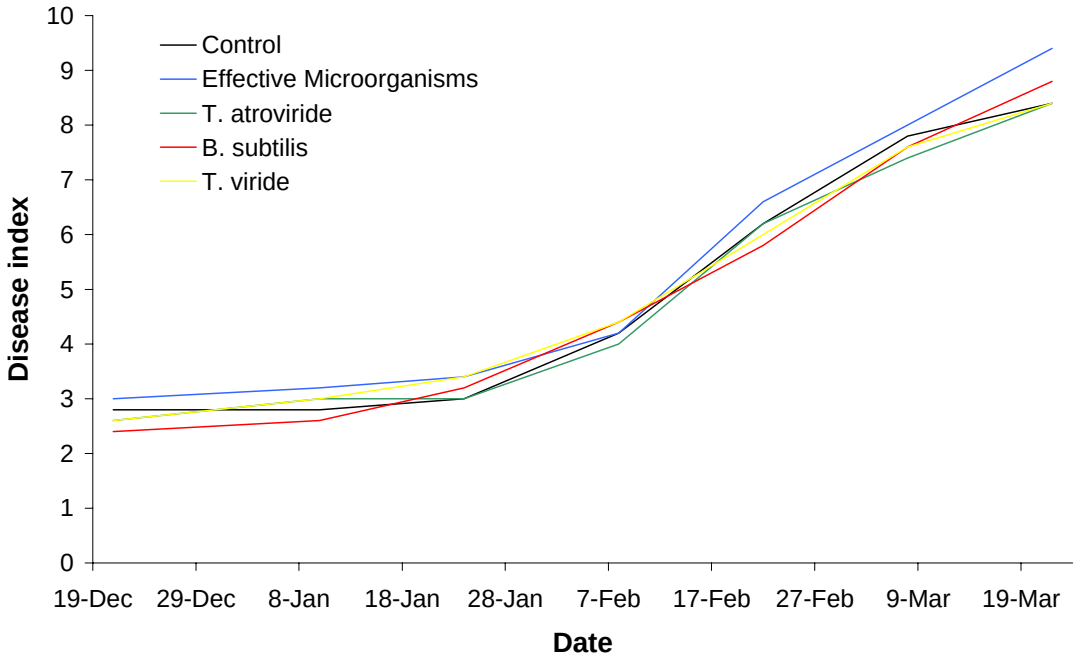

Figure 5.12. Disease symptoms on carrot plants in sprinkler irrigated trial based on a disease index ($LSD_{0.05}$ 0.24 for treatment means).

There was a significant difference in the mean disease index level for all measurement dates between treatments for both sprinkler and tape irrigated trials (Table 5.28). However, the biological differences were minor and all treatments had considerable levels of disease by the end of the trial, with the sprinkler-irrigated trial having a higher disease index with a mean of 5.1 compared to 3.7 for the drip tape irrigated treatment (data can not be compared statistically).

There was no significant statistical or biological difference in seed yield, germination and infestation by *A. radicina* on seed or crop debris for either trial (Table 5.28). Yield was very low, as a result of poor seed set. However, considering the high level of disease seen by visual inspection of the crop, *A. radicina* infestation levels of both seed and crop debris was low. The NaOCl pre-treatment increased the percentage of *A. radicina* detected on seed from 0.5 to 7.7% averaged over both trials (not statistically comparable). No *A. dauci* was detected on untreated seed or crop debris but low levels were found after NaOCl pre-treatment (Table 5.28).

Table 5.28. The effect of four BCA on disease index score, seed yield, germination and *A. radicina* infestation of carrot seed and crop debris as well as *A. dauci* infestation of NaOCl treated seeds. BS = *B. subtilis*, EM = Effective Microorganisms, Ctrl = control, TA = *T. atroviride*, TV = *T. viride*. n/a indicates that $LSD_{0.05}$ and p values are not applicable as no statistical analysis was possible.

Data	Irrigation	Ctrl	BS	EM	TA	TV	LSD	p value
Disease index score (1-10)	**Drip tape**	3.9	3.9	4.1	3.4	3.4	0.24	<0.001
	Sprinkler	5.0	4.9	5.4	4.9	5.1	0.24	0.002
Seed yield (grams m^{-2})	**Drip tape**	1.2	0.6	1.1	0.8	2.0	1.22	0.168
	Sprinkler	0.5	1.0	1.5	1.5	1.6	1.11	0.235
Germination (percentage)	**Drip tape**	49	47	51	52	48	8.1	0.728
	Sprinkler	59	59	62	60	61	8.1	0.936
Infestation (percentage)	**Drip tape**	0.6	0.6	0.4	1.0	0.2	0.96	0.512
	Sprinkler	1.0	0.8	0.2	0.4	0.0	1.00	0.276
Infestation (%) NaOCl treated	**Drip tape**	7.4	8.2	7.3	6.6	7.4	3.9	0.946
	Sprinkler	8.4	6.0	8.2	7.2	10.2	4.1	0.316
Crop debris (percentage)	**Drip tape**	0.0	2.5	0.0	0.0	5.0	n/a	n/a
	Sprinkler	5.0	2.5	0.0	10.0	5.0	n/a	n/a
***A. dauci* (%) infestation**	**Drip tape**	0.6	0.2	0.8	0.0	0.2	n/a	n/a
	Sprinkler	0.0	0.2	0.0	0.0	0.0	n/a	n/a

The main fungal species present on carrot seeds and particularly crop debris were identified as *Colletotrichum* and *Alternaria* spp.

5.8.3. Discussion

The visual disease index showed that the plants had substantial disease symptoms, which increased considerably as the season progressed. There was also a clear visual

difference between the two trials with the overhead-irrigated trial showing more symptoms than the surface irrigated trial. However, the results indicate that the damage was not caused by *A. radicina* and *A. dauci*. While high levels of *A. radicina* and/or *A. dauci* infestation are generally expected to lower seed quantity and quality as a result of reducing the plants' ability to photosynthesise and by directly infesting seeds (Farrar *et al.*, 2004), the infestation levels of *A. dauci* and *A. radicina* found in this study are considered insufficient to cause such low yield and germination levels. Poor pollination could be responsible for poor seed set, however, pollination conditions (weather and proximity to beehives) were considered sufficient.

Colletotrichum gloeosporioides (Penz.) Sacc. and *Alternaria alternata* (Fr.:Fr.) Keissl. have previously been recorded on carrots (Strandberg, 2000). *A. alternata* can infest both foliage and stems and can attack seeds, while *C. gloeosporioides* also attacks umbels (Strandberg, 2000; Farrar *et al.*, 2004). In this study, the *Colletotrichum* spp. were particularly widespread on the surface of the carrot stems forming a continuous black layer with numerous black sclerotia. After incubation, many of the characteristic orange acervuli were formed with long setae on both crop debris and seed. It is considered likely that the *Colletotrichum* spp. were responsible for the visual disease symptoms and defoliation and that this most likely caused the low seed yield and quality.

Even taking into account these unexpected results, there was no biological or statistical difference in levels of *A. radicina* and *A. dauci* among treatments. In addition, the BCA failed to show any controlling effect on the fungal disease complex as a whole, for either the plants or seeds, despite high volume and frequency of BCA application. Thus, in conjunction with the glasshouse-based assay, it appears that none of the BCA tested in this study has the potential to control *A. dauci*, *A. radicina*, *C. carotae* or the other fungal pathogens present on the carrots in their current formulations.

5.8.4. Conclusions

Despite initially promising *in vitro* results the *in vivo* results from both glasshouse and field trials strongly indicate that none of the BCA tested were able to effectively control *A. dauci*, *A. radicina* and *C. carotae* on carrot seed crops. *B. subtilis* (Serenade®) also failed to control *A. dauci*, *A. radicina* and principally *C. carotae* in a fungicide trial on root crop carrots in Michigan, USA (Hausbeck & Harlan, 2003). In an experiment similar to this research, a study of *B. subtilis in vitro* found that it strongly inhibited mycelial growth of *Phytophthora infestans* (Mont.) de Bary, but only achieved less than

20% control in field trials (Dorn *et al.*, 2005). In comparison, other BCA have proved beneficial in controlling carrot fungal pathogens. *Gliocladium virens*, formulated as "Soilguard®" (Thermo Trilogy Co., Maryland, USA) to protect against soil-borne pathogens, was shown to be a mycoparasite of *A. radicina* and to significantly reduce *A. radicina* seed infestation by half on artificially inoculated plants in the field (Coles *et al.*, 2005a, 2005b). However, this result is an exception rather than the rule. Translating successful *in vitro* results into effective foliar active sprays is difficult, which is why there is considerably more research undertaken into using BCA to treat seeds (Schmitt *et al.*, 2004). It is, therefore, currently not considered worthwhile to pursue the use of the BCA tested here for control of carrot fungal pathogens in the field. It may be valuable to study their effectiveness in controlling seed-borne pathogens.

5.9. Levels of *A. radicina* and *A. dauci* on carrot seed commercial crop harvest debris

5.9.1. Methods

On 10 May 2005, samples of crop debris left in the field post harvest were collected from eight locations in the Ashburton area of Canterbury New Zealand: (A) 43°53'048''S, 171°56'592''E, (B) 43°50'595''S, 171°49'522''E, (C) 43°52'074''S, 171°57'373''E, (D) 43°55'145''S, 172°05'079''E, (E) 43°51'174''S, 172°05'079''E, (F) 43°47'080''S, 171°58'200''E, (G) 43°47'426''S, 171°53'363''E, (H) 43°50'007''S, 171°52'109''E. All fields had been managed under non-organic conditions, and approximately two months had elapsed since crops were harvested. Cultivars were unknown although all were F1 hybrid parent lines, i.e. seed as well as pollen parent cultivars were present in each field. Post harvest cultivation practices varied between fields including leaving trash undisturbed or the surface lightly cultivated. For each field, five locations were randomly selected and at each location five 8 cm long pieces of crop stem with diameters between 5-10 mm were collected and sealed in separate polythene bags. Each location was treated as a replicate. Immediately after collection, the stems were tested for the presence of *A. radicina* and *A. dauci* using the International Seed Testing Association seed test for the two pathogens (ISTA, 2005) except that one piece of crop stem was placed in a Petri dish in place of ten seeds. Analysis was carried out by ANOVA on untransformed and square root-transformed data.

5.9.2. Results

Data were normally distributed and the transformation did not alter the results so untransformed results are presented. There was a significant difference ($p<0.001$) in the percentage of *A. radicina* infested stem between fields (Table 5.29). No stems were found to have *A. dauci* infestation. There appeared to be larger numbers of *A. radicina* colonies per stem from fields with higher infestation levels, although these data was not systematically collected.

Table 5.29. Percent infested carrot seed crop debri from eight F1 carrot seed crops ($LSD_{0.05}$ 21.9).

Field	A	B	C	D	E	F	G	H
Percent infested stems	12	88	100	12	80	60	64	36

5.9.3. Discussion

The large variation in percentage of crop debris infested with *A. radicina* was expected as the F1 parent cultivars vary in their levels of resistance. However, *A. radicina* was present in all fields, indicating that the disease is widespread. This has serious implications for the disease management strategy as studies have shown that *A. radicina* can be highly persistent in soils and crop debris (Maude, 1966; Maude & Shuring, 1971; Pryor *et al.*, 1998). The time gap between hosts may be quite short as there are a small range of plants, both crops and weeds, which can act as alternative hosts, some of which are common in Canterbury cropping systems, e.g., carrot root crops and *F. muralis* (Coles, 2003). If *A. radicina* does persist in soils for periods longer than the time between successive susceptible crops or alternative hosts, this may result in soil, crop debris and alternative hosts being an important inoculum source. For example, in California, carrots developed *A. radicina* infestations eight years after the previous carrot crop (Pryor *et al.*, 1998). There is also an overlap of one to two months when current and following years crops are both in the ground. There is no separation distance between successive crops so there is potential for crop debris from the previous year's crop being a source of conidia to infest following crops. This indicates that there is a need to determine prevalence and longevity of *A. radicina*, *A. dauci* and *C. carotae* in agricultural land in general and in the Ashburton area of NZ in particular, ideally correlating these to presence of alternative hosts and rotation and cultivation practices.

5.10. The effect of flame and steam weeding on infestation levels of *A. dauci*, *A. radicina* and *C carotae* in young carrots

5.10.1. Methods

The experiment used a randomised split plot design with four replicates and an untreated control with weeder type as the main plot and pathogen as the sub plot. The flame weeder and steam weeder used here are described in section 4.2.

Carrots were grown in plant raising trays (60 cells per tray; cell volume of 42 cm^3) with one carrot plant per cell, in an unheated glasshouse until foliage was approximately 13 cm high. Conidia to inoculate the carrots were produced and collected by the methods described in section 5.4.2. The number of conidia were adjusted to 4,000 conidia ml^{-1}. Plants were inoculated by spraying 0.5 ml per plant of each pathogen's conidia solution through an airbrush onto the plants. For 48 h, plants were kept in plastic containers (one for each pathogen) with clear lids containing approximately 2 cm of water at 25°C with 12 h dark and 12 h fluorescent light to produce favourable infection conditions for the diseases (Lauritzen, 1926; Strandberg, 1987; Carisse & Kushalappa, 1989, 1992; Carisse *et al.*, 1993). The plants were then removed from the containers and grown in a glasshouse for a further seven days until the proportion of leaf area infested was approximately 30-40% determined according to the disease damage index of Strandberg (1988).

To apply the thermal treatments, six plant-raising trays were buried in a field so that their upper surface was level with the soil surface. The trays were spaced 2 m apart in two lines of four, forming two main plots and four blocks or replicates. The weeders were allocated one main plot each, and then trays within each plot were allocated randomly to each weeder. Three carrot plants, i.e. one per pathogen, were placed in each tray at random to form the sub-plots. Only the trays in the plot to be subjected to the weeders were filled. The weeders were pre-heated for three minutes then driven over the trays at 2 kph, determined by the tractor's speed radar. The treated carrot plants were removed, the weeders switched main plots, fresh carrot plants were placed in the trays assigned to that weeder and the treatment applied. For each disease, four inoculated carrot plants were not subjected to the thermal treatment as controls.

The carrot plants were then grouped again by pathogen and kept spaced apart to reduce cross infestation, with the control plants for each pathogen kept in their own containers. The plants were allowed to regrow for five days in the glasshouse, receiving overhead

irrigation as required. After being placed in the pathogen incubation conditions described above for two days, they were returned to the glasshouse for five more days. This pattern of five days in the glasshouse and two days in the pathogen incubators was repeated for four weeks, during which plants were regularly assessed for infected lesions on the leaves.

5.10.2. Results

No statistical analysis was possible due to abnormal distribution of the data. No re-infestation of the carrot plants was found for any of the pathogens after either thermal treatment, while the disease on the control plants continued to expand for all pathogens, eventually producing complete collapse of the foliage within a week after the first post treatment period under pathogen incubation conditions.

5.10.3. Discussion

The complete elimination of *A. dauci*, *A. radicina* and *C. carotae* from the carrot foliage by using thermal treatment is striking. There is no previous evidence of thermal treatment being used to eliminate pathogens from a growing crop, even though this approach has recently been adopted by the carrot seed industry (Duncan Storrier pers. comm. 2006). It is noted that 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). If seed and root crops are being infested via infested seed, this technique could have the potential to considerably reduce infestation levels by eliminating the pathogen on the crop foliage. The key to more complete disease control would appear to be the role of other sources of inoculum, such as soil, crop debris, alternative hosts and airborne conidia. If these were negligible, i.e. the main source of crop infestation was via infested seeds, thermal treatment could create a break in the diseases' lifecycle and considerably reduce disease levels. Conversely, if there was inoculum present in the soil, the carrot crown or alternate hosts, particularly weeds, or if there was a significant level of airborne conidia, the technique would be less effective, although it may help delay the build up of disease.

This technique can be thought of as a means of breaking the 'green bridge' where a crop is present year round enabling pests or diseases to survive or increase their populations. In carrot seed production this occurs because there is an overlap of four to eight weeks in summer when both the current and following season's crop are growing simultaneously. An example of the potential for 'green bridge' cross-infestation is illustrated by a study in England of *Alternaria brassicicola* on *Brassica oleracea* (L.)

seed crops. For most of the growing season, conidia movement was restricted to within the crop. However, windrowing and particularly threshing released massive amounts of conidia that could be detected up to 1.8 km down-wind and which infested previously uninfested young crops (Humpherson-Jones & Maude, 1982). There is no separation distance practiced in Ashburton between mature and young carrot seed crops, which, coupled with the hot, strong 'NorWester' (Foehn/Chinook type wind) common in Canterbury summers, means that cross-infestation of young crops by conidia released at harvest may well be considerable. Thermal treatment of crops in the after harvest period in autumn, winter or early spring could therefore potentially break crop infestation from both seed-borne infestations and air-borne conidia released at harvest, which could result in a reduction in crop disease levels.

The results of this trial are clearly preliminary as they were obtained under artificial conditions. The situation in the field may be different, especially as in a field situation the carrots will be approximately six months old and have grown through the winter while under controlled conditions they were grown for two months over summer in potting mix. If *A. radicina* can infest the crown and/or root of the carrot plants during the winter, this technique may be less likely to result in complete elimination of disease. As *A. dauci* and *C. carotae* only infest foliage, they should be less likely to survive treatment. Assuming that the soil is free of the pathogens prior to planting, it needs to be determined if infestation from seed or airborne spores is restricted to the foliage or if it spreads to the surrounding soil, for example, because of leaf senescence and decay. If the former is the case, intrarow thermal treatment should be sufficient; otherwise, the entire ground surface may need to be treated, which would result in higher costs. There may also be differences between seed-to-seed crops and transplanted stecklings, the latter of which could be additionally treated with hot water or steam to kill pathogens on the root surface. Further studies are clearly needed to confirm results *in situ*, and to determine the longevity and prevalance of the pathogens in farm soils.

5.10.4. Conclusions

In conclusion, a better understanding of the biology and etiology of *A. dauci*, *C. carotae* and in particular *A. radicina* is required, especially prevalance and persistence in farm soils in the Ashburton region, the levels of airborne conidia both background and around crops, particularly during harvesting operations, and routes of infestation of young crops. In addition, the potential of field scale thermal treatment of crops post

harvest and before significant spring growth to reduce or even eliminate infestations needs to be determined.

Chapter 6. General discussion and conclusions

6.1. Introduction

The three main production issues that were identified for organic carrot seed production, and which formed the basis of this research, were:

- Suitable plant densities for organic crops;
- Weed management, particularly intrarow weeds in overwintered steckling crops;
- Management of fungal pathogens, particularly *A. radicina*.

These issues are interconnected to varying degrees. For example, crop density affects the level of *A. radicina* seed infestation and spring thermal weeding may have potential to control *A. dauci*, *A. radicina* and *C. carotae* as well as certain weeds. Thus, an integrated approach has been chosen to address these issues, which is in keeping with the systems based approach of organic production in general (Kristiansen & Merfield, 2006).

6.2. Crop density

Despite the low yield of cv. A the results show that carrots did not respond in a simple manner to changes in plant density. For example, the yield varied considerably between the cultivars both in amount and response to density, while the proportion of seed produced at each umbel level at different densities was similar for both cultivars. Further, the response of seed quality to density varied between the cultivars for both germination and thousand seed weight (TSW).

Commercial crops are commonly grown at densities around 20 plants m^{-2} in a highly rectangular planting arrangement (Bleasdale & Salter, 1982) with an interrow spacing of 60 cm and an intrarow spacing of approximately 8 cm. This is used because it is believed to improve airflow through the crop canopy in order to reduce relative humidity, which in turn is considered to decrease disease levels (James Smith, pers. comm. 2003). This perception is supported by the results of this trial, which found that seed infestation with *A. radicina* started increasing from density levels of 20 plants m^{-2}. However, the differences in rectangularity mean they are not directly comparable. While these results show that densities higher than 20 plants m^{-2} increased yield and also seed quality, it is suggested that *A. radicina* infestation of seed is potentially more important in terms of overall seed quality.

The highly rectangular spacings used in crop production mean that machinery can access the crop for longer than would be possible on a less rectangular spacing, because there is sufficient open ground between the rows for tractor access even when the crop has started to bolt and increase in size. This extended period of machinery access is particularly important for organic production systems as the main method of weed control is interrow hoeing. Being able to use weeding equipment for as long as possible during crop growth is vital for weed management, particularly of later germinating weeds.

A cropping density of 20 plants m^{-2} using the common 60 cm interrow spacing therefore appears to be the most suitable for production of organic carrot seed crops.

6.3. Weed management

The development of the direct-fired steam weeder has increased thermal weeding options for organic growers and is the first practical alternative to flame weeders. However, the advantages of improved heat transfer, low fire risk, being unaffected by wind and the use of biofuels or diesel as fuel are offset by the greater complexity and capital cost of the steam weeder. It is probable that the larger capital cost will be a deterrent for smaller growers, and that they will purchase less expensive flame weeders despite performance issues. However, for large-scale growers, better performance and reliability may compensate for the higher capital cost of a steam weeder. The prototype clearly needs refinement and the production of commercially available direct-fired steam weeders will be required to determine grower uptake.

6.3.1. Thermal weeding predictive models

The dose response models use by other researchers appear to be of limited use for growers wishing to optimise thermal weed treatment and for making predictions regarding plant susceptibility to thermal treatment. An alternative approach is needed that models the energy transfer of the thermal weeding process and its interaction with plant morphology. This model needs to include the transfer of energy from the heat source onto the plant surface, e.g., by radiation, convection or condensation, how plant morphology and size influence this process and how different types of heat (e.g., flame or steam) affect evapotranspirational heat loss from the plants. Consequently, the conductive heat transfer through different plant tissues (e.g., thin leaves vs. lignified stems) needs to be modelled, which will in turn determine the amount and duration of heat required to kill the hypocotyl stem or the apical and lateral aerial meristems. This appears to be a rather complex exercise and it is unclear how useful it would be as an

aid to growers. The alternative would be to test the potential of the weeder to control a variety of prevalent weeds at a range of speeds in order to determine the optimum working speed for a particular weed stand and thermal weeder. The model approach may, however, prove valuable in a research context and help identify aspects of the heat transfer process that can be improved. This way it might also be possible to gain important insights into how weeds of different morphologies respond to different thermal treatments with the aim of building a general picture and producing more general information that will be of use to growers.

6.3.2. Thermal control of intrarow weeds

The potential for flame or steam weeders to control intrarow weeds in overwintered carrot crops in the spring appears to be limited according to the results of the experiments on thermal treatment of weeds at various growth stages (Section 4.2.3). Most of the weeds requiring control in the spring can be quite large, as they have grown through both autumn and winter. The research presented here showed that most plants could not be killed by thermal treatment even at early growth stages. This indicates that control of overwintered intrarow weeds by springtime thermal treatments is most successful for plants that remain susceptible at larger growth stages, such as *S. media* and *V. agrestis*, which are prostrate, have thin unlignified stems and small leaves that offer little protection to the meristems. Weeds with other morphologies (e.g., rosette) are unlikely to be killed by thermal treatment and may require manual removal if they are considered to negatively affect the crop. It has to be noted that this is an inferred result as no field tests were undertaken. However, as the overwintered weed flora varies across fields and years, results of experiments treating natural weed flora are likely to vary and therefore be of limited predictive value. An understanding of how weed morphology interacts with thermal treatment in order to determine its susceptibility to thermal treatment will allow growers to better predict the success of thermal weeding. They will be able to estimate the potential to control particular weeds in any given crop and what kind of work rate (i.e., energy dose) will be required.

The research on flame weeding carrots at later growth stages (section 4.3) showed that later season (November) thermal treatment had considerable negative effects on seed quality and yield. This indicates that treatment should be undertaken earlier in the season. However, this research has not determined the effects of thermal treatment on carrots at earlier growth stages. Studies on leaf and root trimming stecklings showed that partial foliage removal improved yield (Lal & Pandey, 1986). However, seed-to-

seed crops may react differently, as, unlike seedlings, the stecklings have a period after planting when they are unable to take up water because their new feeder roots have not yet formed. Cutting back the foliage may reduce transpiration from the leaves and reduce stress for the plant, while new roots are being formed, which could result in greater yields. However, the root systems of seed-to-seed crops remain intact as does the plants' ability to absorb soil water. In addition, if healthy photosynthesising leaves are destroyed, root reserves will be drawn on which may set the plant back. Therefore, what is beneficial for a steckling crop may not be advantageous for a seed-to-seed crop. Further, even for the stecklings, complete defoliation had a negative effect, which indicates that it is likely to negatively affect seed-to-seed crops. Clearly, the effect of thermal defoliation of carrot plants in the spring needs to be studied as negative effects of thermal weeding on seed yield and quality could outweigh the reduced costs compared to hand weeding, in which case the technique would be economically questionable. Thermal treatment may produce different results depending on cultivar, planting date and size and whether spring growth has commenced. This means that multiple trials are required to determine the general effect of springtime thermal defoliation on overwintered carrots. As the potential for effective intrarow weed control in springtime appears limited, it does not appear worthwhile to expend resources on researching a technique of such limited value.

Alternative means to reduce the requirement for intrarow hand weeding in spring are still required. Improving the accuracy of interrow hoeing equipment by using machine vision guidance systems appears to have potential. The Garford Robocrop system (Garford Farm Machinery, Market Deeping, England) is now being used in organic carrot seed crops with the aim of reducing the crop gap from 10 cm to 3-4 cm, which means a greater area of the field can be mechanically hoed, thereby reducing hand weeding.

Despite the limited potential for thermal weeders to control weeds, the results of the trials (section 5.10) studying the effect of thermal weeding on fungal pathogens on carrots (*A. dauci*, *A. radicina* and *C. carotae*) indicate that autumn, winter or springtime thermal treatment of overwintered carrot plants could play an important role in an integrated pathogen management system. In this case it would be worthwhile establishing the effect of thermal treatment in autumn, winter or spring on carrot seed yield.

6.4. Pathogen management

6.4.1. Biological control agents

The failure of the biological control agents (BCA) *R. rhizogenes*, *B. subtilis*, *T. atroviride*, *T. viride* and Effective Microorganisms (EM) to control the pathogens was disappointing but not entirely unexpected. The use of a progression of tests from *in vitro* to *in vivo* with both glasshouse and field trials means that the results can be considered reasonably conclusive. The *in vitro* tests provided highly controlled conditions where the direct interaction of pathogens and the BCA could be studied for any inhibitory effects against the fungal pathogens. The BCA compatibility testing substantiated the BCA vs. pathogens results. *B. subtilis* inhibited the fungus *T. viride* just as it had the fungal pathogens, while *R. rhizogenes* failed to inhibit the pathogens or *T. viride* but was in turn inhibited by *B. subtilis*. The benefit of the controlled conditions used in the *in vitro* tests can also be regarded as a weakness in that conditions were used that are highly uncharacteristic of the natural environment in which control of the pathogens is required. The glasshouse tests, therefore, provided a more realistic test situation. However, they were still unrealistic in a number of ways including the inundative application of pathogen conidia to the carrot foliage, the provision of optimal conditions for infestation and the use of young carrot plants when, in the field, the pathogens are most problematic on older plants. The glasshouse tests also provided more hospitable conditions for the BCA to survive as the glasshouse protected them from the weather. The semi-controlled conditions allowed the BCA to be introduced to the foliage before the pathogens rather than trying to kill the pathogen once infestation had occurred. This is expected to give them an advantage, as they only have to prevent the pathogen conidia germinating and infesting the carrot. This would particularly be an issue for *T. atroviride* as it is a resource competitor, unlike *T. viride* which is a mycoparasite (Karthikeyan *et al.*, 2003; Stewart *et al.*, 2005; van Toor *et al.*, 2005). The lack of control of any of the pathogens by the BCA was problematic and could have been a result of experimental design rather than the actual inability of the BCA to control the pathogens in the field. For example, if *B. subtilis* had shown strong control of *A. dauci* but lesser control of *A. radicina* on the carrots, paralleling the *in vitro* tests, it would have been reasonable to have confidence in the validity of the two tests. However, there were no consistencies in the results of the *in vitro* and glasshouse *in vivo* tests, which meant the conclusions had to be more circumspect. The field trial found no difference among the treatments, which was in agreement with the glasshouse trials. These two identical results, obtained under quite different experimental conditions

indicate that the results are robust. Further research on these BCA for control of fungal pathogens on carrot plants, therefore, appears unwarranted.

The domination of the fungal flora on the carrots by fungal pathogens such as *Colletotrichum* spp. and *A. alternata* observed in the field experiment raises the issue of whether *A. dauci*, *A. radicina* and *C. carotae* are the only or even most important pathogens of carrots in the Canterbury area. Both genera were also present on seed, both untreated and 5% NaOCl surface sterilised, indicating they were not only present on the seed surface but that they may have penetrated the seed. However, as cultivar A680059 is considered highly susceptible to *A. dauci*, *A. radicina* and *C. carotae*, it may also be susceptible to other fungal pathogens and as such not representative of other cultivars. Nonetheless, results suggest that *A. radicina* may not be the only pathogen causing production problems and better identification of the fungal pathogen flora of carrot seed crops in Canterbury is necessary, especially if they are seed borne.

Despite the lack of efficacy in the field, the BCA may still have some potential for the control of the pathogens on seed. For example, one of the studies that indicated that *B. subtilis* may have potential to control the pathogens found that the antibiotics it produces were effective as a seed treatment (Besson & Michel, 1987). Treating seed with the BCA could be done under controlled conditions optimised for the BCA, giving them a better chance of controlling the pathogens. This could potentially address problems that arise when *A. radicina* seed infestation rates increase considerably between harvests and when seed is shipped to Europe (Jo deFilippi, pers. comm. 2006). Seed is normally harvested by combine harvesters, which are unable to effectively separate the awned carrot seeds from the rest of the harvest trash. This results in the entire small harvest fraction, including crop stems and weed seed, being retained with the seed. The seeds are then separated by specialist static carrot seed cleaning equipment, but there can be delays of up to three months between harvest and cleaning due to the amount of harvest material to be processed and the capacity of the machines. During this time, *A. radicina* infestation levels are known to significantly increase. It would be of considerable benefit for seed producers, if the BCA could suppress *A. radicina* and/or other pathogens in the seed and trash mixture. The use of BCA to treat seeds appears to be more successful than their use as crop sprays. For example, biopriming of infected carrot seed with *Clonostachys rosea* (Link: Fr.) Schroers *et al*. controlled *Alternaria* spp. as effectively as iprodione (Jensen *et al.*, 2004).

The lack of effectiveness of the BCA, and the general difficulties in finding BCA that are effective against fungal pathogens on growing crops means that alternative management approaches for *A. dauci*, *A. radicina* and *C. carotae* are required.

6.4.2. Thermal control

As discussed in section 5.10.3, thermal treatment has the potential to break the pathogens' lifecycle. Although no other examples of thermal control of pathogens on living plants have been found in the literature, there are biological techniques that are effective at breaking pathogens' lifecycles, resulting in reduced disease levels on subsequent crops. For example, in New Zealand apple orchards, it is common for sheep to be intensively grazed over winter to the point that they eat the dead apple leaves on the ground, which destroys the resting stage of the blackspot fungus (*Venturia inaequalis* (Cooke) Wint.) on the leaves. A similar approach is being used in current research on *B. cinerea* in grapes by applying a range of biological mulches and composts under the vines, which speeds up the decomposition of the diseased plant tissues on the ground, reducing *B. cinerea* levels in following crops (Marco Jacometti, pers. comm. 2006). This indicates that using a thermal treatment of carrot crops to break the pathogens' lifecycle could result in lower disease levels later in the year.

Further research is clearly required to determine the efficacy of such techniques in the field, and also to determine the infestation routes of crops and their relative contribution to both crop and seed infestation levels, e.g., via F1 parent seed, cross contamination at harvest, crop debris in neighbouring fields, alternative hosts or contaminated soil. If autumn, winter or spring thermal weeding can disinfest a field or reduce disease levels to very low levels, it will be of limited use if the field is re-infested from other sources later in the season.

Despite the clear need for further research, this technique is now being actively used by Bejo Zaden in Europe, and Midlands Seed Ltd. who are planning to use a flame weeder to treat the intrarow area of as many of their carrot crops as possible, starting in spring 2006 (Jo deFilippi, pers. comm. 2006).

If the technique proves to be effective at reducing pathogen levels, it will be worthwhile to improve its energy efficiency. The research on flame weeding (section 4.2.3) shows the difficulty of destroying all the leaves on larger plants due to shading and volume of foliage. Overwintered carrot stecklings' foliage can vary from less than 5 cm up to 20 cm depending on the season (Jo deFilippi, pers. comm. 2006). This could equate to a considerable weight of foliage, which would require a large amount of energy to heat,

resulting in high costs and/or low work rates. If the pathogens are confined to the carrot foliage, the idea of mechanically removing most of the foliage with an agricultural mower prior to thermal treatment as proposed in section 04.2.3.3 could reduce costs and improve work rates. It has to be taken into consideration that shredded foliage distributed across the field by the mowing process could act as a source of re-infestation. However, if the distribution of small pieces of foliage happened evenly across the soil surface in a thin layer, it may be easier to treat than were it still on the carrot plant. Alternatively, it may be more cost effective to build a machine to cleanly cut the foliage into collection bins and remove from the field leaving only the crown of the carrot requiring thermal treatment. There are a large number of assumptions underlying these suggestions so this is an area where considerable further research is required.

6.4.2.1. Thermal seed treatment

Even if thermal disinfestation of carrot plants in spring is effective, it is unlikely to completely eliminate the pathogens from the crop for the whole season, so infested seed may still be produced. Hot water treatment of such seeds could be useful to improve seed health and the health of subsequent root crops. It may also be beneficial to treat seed with BCA after heat treatment to further improve seed quality. The benefits of combining the two treatments clearly requires empirical validation.

Hot water treatment of the F1 parent plant seed may also be a valuable part of an integrated pathogen management strategy for seed crops. This would depend on the relative contribution of infested F1 parent seed, cross contamination at harvest, and other infestation sources to field infestation levels. If, for example, cross contamination at harvest accounts for nearly all the disease in the field and the seed produced, then parent seed treatment is unlikely to noticeably decrease infestation levels. However, infested parent seed would introduce the pathogens into the crop at sowing, four to six weeks before harvest operations may release airborne conidia. This difference is important as the seed infestation route may be more likely to result in root infestation by *A. radicina*, which may be more difficult to treat than foliar infestation. If so, this would mean heat treatment of F1 parent seed would be valuable. This may be a potentially useful avenue of research.

6.5. Conclusions

A suitable crop density for organic carrot seed crops is around 20 plants m^{-2} as higher densities have the potential to increase *A. radicina* seed infestation levels. This spacing

and a rectangular layout with 60 cm interrow spacing suit organic production because they allow increased machinery access time compared with a less rectangular designs.

The BCA tested in this study were found to be ineffective at controlling the pathogens on carrot plants under field conditions. However, they may still have potential for the treatment of seeds and the mix of seeds and trash produced by field harvest before they are processed. The BCA and hot water treatment may also be valuable for treating cleaned seed before it is shipped to Europe, rather than treating it on arrival.

Although thermal weeders appear to have limited potential to control weeds, they appear to have greater potential to manage *A. dauci*, *A. radicina* and *C. carotae* as part of an integrated pathogen management program. This will still leave intrarow weeds to be manually controlled but the improved accuracy of computer vision guidance systems for interrow hoes and the reduced crop gap that this permits means that hand weeding should be less of a problem than when organic carrot seed production was first pioneered in New Zealand.

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Appendixes

Appendix A. Illustrations of the Atarus Stinger® and VaporJet H1200® steam weeders

Figure A.1. Photo of Atarus Stinger®, © D.J. Batchen Pty. Ltd., Auburn, New South Wales, Australia.

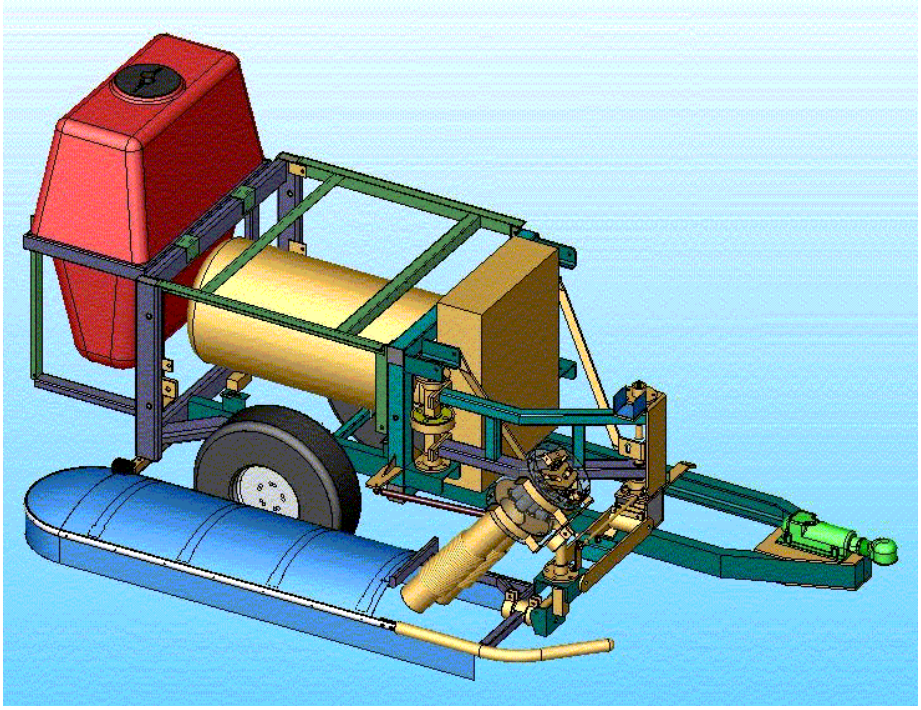

Figure A.2. Diagram of Atarus Stinger®, © D.J. Batchen Pty. Ltd., Auburn, New South Wales, Australia.

Figure A.3. Photo of the VaporJet H1200® steam weeder. © Thermal Options Ltd.