Project title	Reducing disease losses in carrots and parsnip crops through appropriate seed health standards
Project number:	FV 325
Project leader:	Dr J E Thomas, NIAB
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Grower Summary

Headline

- The health status of carrot seed tested was high with no recordable disease observed on carrot foliage or roots in field plots grown from the samples, even where low levels of Alternaria species were seen.
- In parsnip seed, levels of Itersonilia were sometimes very high, but no other pathogens were seen at significant levels. There was no indication that canker levels on roots were greater when grown from seed with high infection therefore a relatively high threshold on seed is likely to be safe in commercial production.

Background and expected deliverables

Carrot and parsnip seed may be infected with a number of seed-borne pathogens which have the potential to cause disease early in the life of the crop, and contribute significantly to later epidemic progress. Carrot and parsnip producers have been concerned that seed supplies carry significant disease and that this is responsible for subsequent problems. This project is aimed at examining samples of seed used by producers and identifying any health problems, and then investigating the relationship between seed health status and disease developing in the early stages of the growing crop. The results will deliver the basis for a voluntary seed health assurance by identifying infection levels which are likely to give rise to disease in the field, and those which are not.

Summary of the project and main conclusions

Carrot seed samples were obtained during the first year of the project, but only one parsnip seed sample was received. A project extension enabled 43 parsnip samples to be evaluated in seed tests and field plots over two years, and a total of 57 carrot seed samples in three years. Nearly all the carrot samples were completely free from disease using standard testing techniques and would be unlikely to be the cause of field problems. Samples with up to 3% *Alternaria dauci* did not give rise to detectable disease in field plots and this would therefore appear to be an acceptable level in seed. Irrigation during establishment and subsequent growth, coupled with the relatively wet years during which the work was undertaken, maximised chances of transmission from seed to plant. A threshold of 5% for combined *Alternaria dauci* and *Alternaria radicina* is proposed as a voluntary standard which is both safe and achievable.

The health status of the parsnip seed samples was very variable, with several samples having around 50% infection with *Itersonilia pastinacae*, one of the main causal agents of canker. Some of the high infection samples had been supplied to growers, but most commercial seed had around 20% infection or less. Seed was obtained from three major parsnip suppliers in the UK, with some being given for experimental purposes only to aid the understanding of the relevance of seed infection. Samples were also obtained direct from growers. Itersonilia was the only pathogen found at significant levels. It was recovered at a low frequency from juvenile plants in one season only (see Table 1). Plants showed a range of symptoms, including blackened crowns and petioles. However, there was no proportional relationship between incidence on seed and the incidence of Itersonilia symptoms and re-isolation. Black and brown cankers (Fig. 1) developed by October/November in each year, and were still evident in January. Itersonilia was recovered more frequently from black than brown symptoms, though the cause of the majority of brown symptoms remains unknown. Not all black cankers gave rise to Itersonilia, and may have had other causes, or secondary organisms may have suppressed Itersonilia on agar plates. As with juvenile plants, there was no significant positive relationship between either the incidence of black cankers or the incidence of isolated Itersonilia, and the original level of infection on the seed (see Table 2 for example from January 2010 harvest).

Fig 1. Black and brown cankers

Table 1: % of juvenile plants with Itersonilia compared to % infection on the seed, July 2008

Sample	% plants with Itersonilia	% seed infection with Itersonilia
PNP 174	0.0	12.5
PNP 130/54	4.4	4
PNP 130/09	2.2	4.5
PNP 190/27	5.5	9.5
PNP 190/36	0.0	0
PNP 10/09	7.8	23.5
PNP 10/06	2.2	48
PNP 173/46	2.2	4.3
PNP 173/93	1.1	0
PNP 150/72	1.1	0
PNP 150/13	0.0	10.5
GLADIATOR	0.0	0
COUNTESS	0.0	0
POLAR	1.1	0
PALACE	0.0	0
55517	2.2	8.7
PH 8	0.0	0
PALACE F1/79	0.0	0
PALACE F1/80	1.1	8.6
POLAR F1/82	0.0	0.7
PH 9	1.1	12.9
DUCHESS	5.6	0

Table 2: % of plants with black cankers and % of plants with Itersonilia, compared to % infection on seed, January 2010

Sample	% black crowns	% roots with Itersonilia	% seed infection with Itersonilia
E15024	14.0	1.5	8.6
E15232	10.8	1.5	7.2
E16042	8.4	0.3	10.5
E14956	10.9	0.0	20.5
E15237	14.6	1.2	2.0
E15011	9.4	3.6	24.7
E15374	4.4	0.0	54.0
E14600	15.5	1.3	10.8
E15375	9.8	0.6	50.8
E15171	1.4	0.0	46.2
E15470	6.4	0.5	2.0
E15249	13.4	0.9	12.5
E15373	15.6	2.4	51.5
E15410	5.0	0.3	20.0
E15339	17.8	3.6	0.0
E15201	18.0	3.3	14.0
1	4.5	0.0	23.5
2	7.7	0.4	19.5
3	10.9	4.1	13.5
2008 re-test	25.4	2.3	1.1

These results show that while Itersonilia on seed can introduce infection into a crop, there is no evidence to indicate that high seed infection levels lead to high canker levels. Transmission from seed to plant must occur, since there was no recent parsnip material close to the experimental site. Subsequent spread in the trial is most likely to have occurred sporadically through spore discharge from initial infected material, but a proportional relation between seed infection and plant infection was not seen.

The absence of this relationship for Itersonilia suggests that a relatively high threshold on seed may be tolerated with 20% infection being suggested as an acceptable level. In some seed production seasons, even this level may be hard to achieve, but the evidence obtained here suggests that levels up to 50% are still safe for commercial production, with the proviso that a nil level is still highly desirable for preventing introduction of disease to a production area.

Main Points:

- The health status of 57 samples of commercial carrot seed tested over three years was very high. Only trial samples had any *Alternaria dauci* or *Alternaria radicina*, and the highest level recorded was 3% (*A. Dauci)*. There was no *Cercospora carotae* present. Not all samples were tested for *Xanthomonas hortorum*, but two selected samples were negative.
- There was no evidence of fungal or bacterial disease in field plots established from any of the samples.
- A combined threshold of 5% seed infection with Alternaria species is suggested as presenting a low risk of causing a disease problem in commercial production. The health status of 43 parsnip samples was very variable, with some seed lots having between 20% and 50% infection with *Itersonilia pastinacae*, the causal agent of parsnip canker. Some of these were supplied to growers; others were obtained from various suppliers for experimental purposes. The incidence of other potential pathogens on the seed obtained was very low.

- There was no indication that high levels of Itersonilia on seed resulted in proportionally higher levels of parsnip canker.
- A nil level of Itersonilia on seed will not guarantee freedom from disease in the crop if other sources of infection are present (soil/debris/nearby crops)
- Seed infection may introduce low levels of infection into disease free areas, but high levels on seed do not appear to be a cause of a high incidence of canker. A threshold of 20% infection is suggested as presenting a low risk of canker in commercial production.

Financial benefits

Carrot seed samples were obtained from seven seed houses, and represented most major interest varieties and newer ones. Growers can therefore be assured that the health status of the majority of carrot seed surveyed is high, and unlikely to initiate extensive field disease development. Parsnip seed samples were obtained from two major suppliers and from growers where the supplier was unknown. Though the health status of the seed was very variable for Itersonilia infection, there was no link between the higher levels and high canker incidence. Seed is thus unlikely to be responsible for serious canker, and appropriate management of the growing environment (rotations, hygiene and fungicide use and variety choice) is of greater importance in reducing field incidence of the disease.

Voluntary standards of health for carrot and parsnip seed have been suggested here. In practice, these standards will usually be met and exceeded and seed will not be a major cause of disease in a crop.

Action points for growers

- Ask for seed health test results and compare to the standards suggested here.
- Disease like symptoms may appear on juvenile plants, but if seed was healthy, the symptoms are unlikely to be caused by seed-borne disease.
- Monitor any disease-like symptoms carefully for further spread. Manage crops to reduce diseases such as Alternaria on carrots and Itersonilia on parsnips and prevent the establishment of reservoirs of inoculum.

Science Section

Introduction

Carrot and parsnip seed may be infected with a number of seed-borne pathogens which have the potential to cause disease early in the life of the crop and contribute significantly to later epidemic progress. Furthermore, seed-borne disease may introduce problems into relatively "clean" areas and create a reservoir of inoculum. Organic seed is particularly vulnerable to the effects of seed-borne disease, and in conventional seed, available seed treatment products may not always offer complete control of a range of diseases. A recent small survey (see FV 261) on parsnip seed confirmed the presence of various diseases in seed tests, but did not relate these to disease in the field. Carrot growers have frequently suspected that seed carries significant disease, but there has been no attempt to relate this experimentally to subsequent disease development. Relating disease outbreaks to seed-borne origins is usually difficult, since many growing areas have a high intensity of crops or crop debris which perpetuates diseases. Nevertheless, it is vital that both growers and suppliers of seed have information on seed health, and an indication of whether certain levels of disease may contribute significantly to disease development or not. This project aims to test seed lots sold in the UK and investigate the relationship between test result and disease developing during the early stages of crop growth. The results obtained will then be used to provide the basis for a voluntary assurance of health test results.

Materials and methods

Requests for carrot and parsnip seed samples used by growers were made through the BCGA group, and through the project coordinator. In addition, commercial samples used to drill the BCGA carrot demonstration trial were included. A total of 26 carrot samples were examined in the first year of the project but unfortunately only one parsnip sample was obtained (see FV 325 first year report). The project was extended to allow two seasons data to be obtained on parsnip. A further 21 carrot seed samples were obtained in 2008, together with 22 parsnip samples. The carrot samples were commercial lots used in BCGA demonstration trials and parsnip seed lots contained both samples supplied to growers and further samples provided by seed merchants with a range of infection levels. The 2008 experiment was drilled on 22nd May. A further 21 samples of parsnip were drilled on 13th May 2009. The opportunity was also taken in 2009 to establish a third carrot trial, with 10 samples from BCGA demonstration material. In each year, plots were 2 rows x 9 m long, with three replications per sample. Carrot and parsnips were sown as separate trials. All work was carried out on the NIAB trial ground in Cambridge, well isolated (at least 5km) from any commercial carrot or parsnip production. The 2008 and 2009 trial sites were separated by just over 1 km. Plots were irrigated to promote establishment, and to provide a favourable environment for disease development (25 mm per week over two or three 1 h periods on separate days depending on rainfall). Plots received standard commercial practice for herbicides and fertiliser, but no fungicides or insecticides.

Whole plants were sampled on the dates shown in Table 1. Groups of five plants within rows were removed. Sample number was either 30 or 50, except for final samples from the parsnip experiments when all remaining roots were removed. Leaves, petioles and roots were rinsed carefully to remove soil and examined visually for signs of disease. Suspect samples for various diseases were surface sterilised in 5% sodium hypochlorite and plated on malt extract agar (for Alternaria spp.) or potato dextrose agar (all other organisms) for colony identification. At each sampling time, sections of tissue from at least 20 healthy parsnip roots, i.e. no visual symptoms, or petiole and foliage sections at the early lift, were also plated to check for presence of Itersonilia. Any significant foliar disease was also assessed visually in the field on a whole plot basis, and the causal agent confirmed by examination of spores from leaf lesions.

Table 1: Sampling times*

	Parsnip		Carrot	
	2008 trial	2009 trial	2008 trial	2009 trial
Sample 1	14th July	8th July	21st July	20th July
Sample 2	10th November	23rd October	10th November	4th November
Sample 3	19th January (2009)	18th January (2010)	23rd February (2009)	Omitted

* sampling commenced on dates shown and was completed within 10 days

Sub samples of the seed supplied were retained for testing in the laboratory. 200 seeds of each were plated on potato dextrose agar, or incubated in blotter tests, and assessed for the presence of known pathogenic agents, and any other organisms present. Seed tests were carried out either just before or within one month of drilling. For known pathogens, standard identification methods available in ISTA/ISHI protocols were used. Treated seeds were plated without removing treatment.

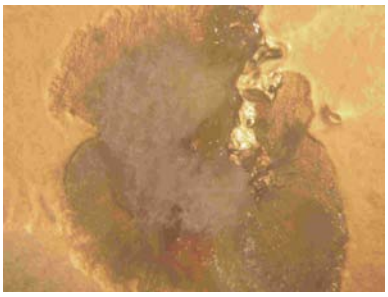
Results

a) Parsnip 2008 experiments
Seed health test results are shown in Table 2. There was considerable variation in levels of Itersonilia on seed, but levels of Alternaria species and Phoma species were very low.

Table 2: Incidence of pathogenic fungi in parsnip seed samples

		% infection			
Sample Code	Source	*Alternaria dauci*	*Alternaria radicina*	*Itersonilia pastinacae*	*Phoma* sp
PNP 174	Seed company	0	0	12.5	0
PNP 130/54	Seed company	0	0	4	0
PNP 130/09	Seed company - not commercial	0	0	4.5	0
PNP 190/27	Seed company	0	0.5	9.5	0.5
PNP 190/36	Seed company - not commercial	0	0	0	0
PNP 10/09	Seed company	0	0	23.5	0
PNP 10/06	Seed company - not commercial	0	0.5	48	0
PNP 173/46	Seed company - not commercial	0	0	4.3	0
PNP 173/93	Seed company	0	0	0	0
PNP 150/72	Seed company - not commercial	0	0	0	0
PNP 150/13	Seed company	0	0	10.5	0
GLADIATOR	Grower	0	0	0	0
COUNTESS	Grower	0	0	0	0
POLAR	Grower	0	0	0	0
PALACE	Grower	0	0	0	0
55517	Grower	0	0	8.7	0.5
PH 8	Seed company	0	0	0	0
PALACE F1/79	Seed company	0.5	0	0	0.5
PALACE F1/80	Seed company	0	0	8.6	0
POLAR F1/82	Seed company	0	0	0.7	0
PH 9	Seed company	0	0	12.9	0
DUCHESS	Seed company	0	0	0	0

The level of symptoms on various plant parts giving rise to Itersonilia, on juvenile plants in July, was very low (Table 3). A typical colony on agar of *I. pastinacae* recovered from plants is shown in Fig 1, together with diagnostic ballistospores.



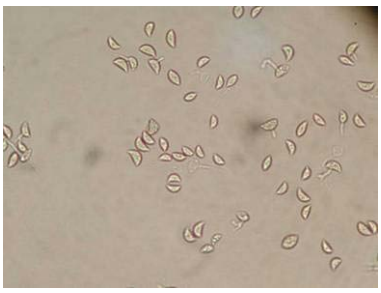


Fig 1: Typical Itersonilia colony on agar plate and ballistospores

A range of plant parts was infected. Symptom type on infected parts varied in colour from black to reddish brown lesions. Plant parts with no suspect symptoms did not produce any Itersonilia. The numbers of plants with suspect symptoms which did not give rise to Itersonilia was extremely low, and data are not presented.

Table 3: Mean % of plants out of 30 infected with Itersonilia on various tissues with different symptom types, July 2008

	Black crowns	Brown cotyledons	Root tip - orange	Leaf tip black	Leaf tip brown	Red brown petiole	Brown petiole	Total % Itersonilia
PNP 174	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PNP 130/54	4.4	0.0	0.0	0.0	0.0	0.0	0.0	4.4
PNP 130/09	2.2	0.0	0.0	0.0	0.0	0.0	0.0	2.2
PNP 190/27	4.4	0.0	0.0	0.0	1.1	0.0	0.0	5.5
PNP 190/36	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PNP 10/09	6.7	0.0	0.0	0.0	0.0	1.1	0.0	7.8
PNP 10/06	0.0	0.0	0.0	0.0	1.1	0.0	1.1	2.2
PNP 173/46	0.0	0.0	0.0	0.0	2.2	0.0	0.0	2.2
PNP 173/93	0.0	0.0	0.0	0.0	0.0	0.0	1.1	1.1
PNP 150/72	0.0	0.0	0.0	0.0	1.1	0.0	0.0	1.1
PNP 150/13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
GLADIATOR	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
COUNTESS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
POLAR	1.1	0.0	0.0	0.0	0.0	0.0	0.0	1.1
PALACE	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
55517	1.1	0.0	1.1	0.0	0.0	0.0	0.0	2.2
PH 8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PALACE F1/79	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PALACE F1/80	0.0	0.0	0.0	1.1	0.0	0.0	0.0	1.1
POLAR F1/82	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PH 9	1.1	0.0	0.0	0.0	0.0	0.0	0.0	1.1
DUCHESS	0.0	3.3	0.0	0.0	2.2	0.0	0.0	5.6

At the November harvest, some severe black crown cankers were recorded together with black streaking or shorter, thinner black stripes (Fig 2).

Fig 2: Black crown cankers and black streaks

The percentage of roots showing these symptoms is shown in Table 4. Target root number sample per plot was 50, though this declined in some plots due to poorer establishment. Not all of the black symptoms gave rise to Itersonilia when isolated. Table 4 also shows the total % of roots sampled which produced Itersonilia for each symptom type and the total % over all symptom types.

Table 4: Mean % of roots showing canker like symptoms and mean percentage giving rise to Itersonilia colonies, November 2008

	Mean root no.	% with black crowns	% with black streak	% with black stripe	% Healthy roots (no symptoms)	% crowns infected	% streaks infected	% stripes infected	Total % Itersonilia
PNP 174	49.0	7.5	3.4	0.0	89.1	1.0	0.0	0.0	1.0
PNP 130/54	51.3	4.5	5.8	1.3	88.3	0.6	1.0	0.0	1.6
PNP 130/09	45.3	7.3	5.1	0.0	87.5	0.0	0.0	0.0	0.0
PNP 190/27	49.7	2.7	4.0	0.0	93.3	0.0	0.0	0.0	0.0
PNP 190/36	52.3	4.5	5.1	0.0	90.5	0.0	0.0	0.0	0.0
PNP 10/09	35.3	8.5	8.5	1.9	81.1	0.0	0.0	2.8	2.8
PNP 10/06	57.7	8.7	21.4	1.7	68.2	0.0	2.9	0.0	2.9
PNP 173/46	53.0	8.8	20.1	0.0	71.1	0.0	0.0	0.0	0.0
PNP 173/93	51.0	2.6	4.6	0.6	92.2	1.0	0.6	0.0	1.6
PNP 150/72	50.3	4.0	5.3	1.3	89.4	1.0	0.7	0.0	1.6
PNP 150/13	33.0	1.0	4.0	0.0	94.9	0.0	0.0	0.0	0.0
GLADIATOR	20.0	10.0	13.4	3.4	73.3	2.5	0.0	0.0	2.5
COUNTESS	35.0	6.7	2.9	0.9	89.5	1.9	0.0	0.0	1.9
POLAR	37.0	6.3	10.8	1.8	81.1	2.7	0.0	0.0	2.7
PALACE	33.0	3.0	13.1	3.0	80.8	0.0	0.0	0.0	0.0
55517	53.7	5.6	8.1	2.5	83.9	0.0	0.0	1.9	1.9
PH 8	52.3	1.3	2.5	1.3	94.9	1.9	0.0	0.0	1.9
PALACE F1/79	55.7	5.4	7.8	0.0	86.8	0.0	0.9	0.0	0.9
PALACE F1/80	35.7	6.5	3.7	0.0	89.7	1.4	0.0	0.0	1.4
POLAR F1/82	41.7	4.8	10.4	1.6	83.2	1.2	1.2	0.0	2.4
PH 9	32.3	0.0	2.1	1.0	96.9	0.0	0.0	0.0	0.0
DUCHESS	20.0	0.0	13.4	1.7	85.0	0.0	0.0	0.0	0.0

At the final harvest in January/February 2009, all remaining roots in each plot were lifted. Black crown and shoulder symptoms were still visible, but more brownish areas of rotting were evident (Fig 3). These were scored separately. Root shanks were free of black or brown symptoms and showed only occasional root fly damage. Table 5 shows the % of roots with each symptom type, and the percentage of total root numbers which gave rise to Itersonilia colonies. The majority of Itersonilia was associated with black crowns and shoulders and very little with brown symptoms.

Fig 3: Brown cankers, January 2009

Table 5: Mean % of roots with black or brown crown and shoulder symptoms, and percentage with Itersonilia from black and brown symptoms, January-February 2009

	Mean root number	% with black crowns only	% with black shoulders only	% with Itersonilia (of total roots)	% with brown crowns only	% with brown shoulders only	% with Itersonilia (of total roots)
PNP 174	237.3	0.6	0.7	0.0	0.7	0.0	0.0
PNP 130/54	212.3	0.8	1.7	0.0	0.0	0.0	0.0
PNP 130/09	199.3	0.2	0.2	0.0	0.2	0.0	0.0
PNP 190/27	201.0	1.3	1.7	0.3	0.3	0.3	0.0
PNP 190/36	227.3	0.9	0.4	0.1	0.6	0.0	0.0
PNP 10/09	187.3	1.6	2.7	0.0	1.1	0.0	0.0
PNP 10/06	171.3	1.6	1.2	0.0	1.6	0.0	0.0
PNP 173/46	180.3	0.6	1.5	0.2	1.1	0.2	0.0
PNP 173/93	193.7	1.9	0.9	0.0	0.9	0.0	0.0
PNP 150/72	198.7	0.3	1.0	0.2	0.2	0.2	0.0
PNP 150/13	201.7	1.0	1.7	0.2	0.7	0.2	0.0
GLADIATOR	70.0	1.4	0.0	0.0	0.0	0.5	0.0
COUNTESS	49.0	4.1	4.1	1.4	0.0	0.0	0.0
POLAR	58.3	1.7	4.0	0.0	0.6	0.0	0.0
PALACE	70.7	2.8	2.4	0.0	1.9	0.0	0.0
55517	139.3	1.0	1.7	0.0	0.0	0.0	0.0
PH 8	209.0	0.0	0.0	0.0	0.0	0.0	0.0
PALACE F1/79	230.0	2.0	1.5	0.1	0.9	0.1	0.0
PALACE F1/80	225.3	0.3	0.4	0.0	0.1	0.3	0.1
POLAR F1/82	215.3	1.1	0.8	0.5	0.3	0.0	0.0
PH 9	196.0	0.3	0.2	0.0	0.0	0.0	0.0
DUCHESS	36.0	0.9	0.9	0.0	0.9	0.0	0.0

There was no significant relation between the incidence of Itersonilia in the seed samples and the incidence of either canker-like symptoms on the roots, or the number of canker symptoms from which Itersonilia was recovered at any of the sampling times (see Fig 4 - 8).

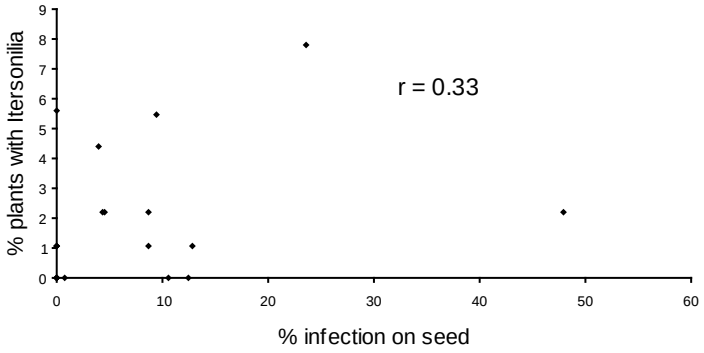


Fig 4: % juvenile plants with Itersonilia and % seed infection, July 2008

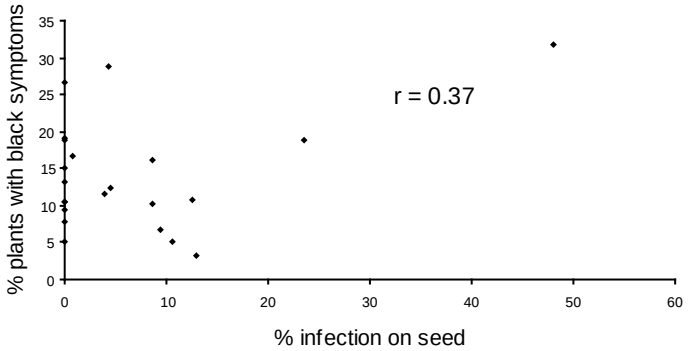


Fig 5: % plants with black symptoms and % seed infection, November 2008

Fig 6: % roots infected with Itersonilia and % seed infection, November 2008

Fig 7: % roots with black crowns and shoulders and % seed infection, January 2009

Fig 8: % roots with Itersonilia and % seed infection, January 2009

b) Parsnip 2009 experiment

Samples obtained in 2009 again had variable levels of Itersonilia infection, but only a low level of Phoma in one sample (Table 6). There was no *Alternaria dauci* or *Alternaria radicina* in any sample. One sample was a re-test of PNP 10/09 from 2008.

Table 6: Incidence of pathogenic fungi in parsnip seed samples

Sample code	Source	% Itersonilia	% Phoma
E15024	Seed company	8.6	0.5
E15232	Seed company	7.2	0
E16042	Seed company	10.5	0
E14956	Seed company	20.5	0
E15237	Seed company	2.0	0
E15011	Seed company	24.7	0
E15374	Seed company	54.0	0
E14600	Seed company	10.8	0
E15375	Seed company	50.8	0
E15171	Seed company	46.2	0
E15470	Seed company	2.0	0
E15249	Seed company	12.5	0
E15373	Seed company	51.5	0
E15410	Seed company	20.0	0
E15339	Seed company	0.0	0
E15201	Seed company	14.0	0
1	Commercial	23.5	0
2	Commercial	19.5	0
3	Commercial	13.5	0
2008 re-test	Seed company	1.1	0

At the July sampling time, a range of black or brown areas, spots or streaks were observed on cotyledons, leaf tips, petioles, roots and crowns. The % of affected plant parts is shown in Table 7. A number of non-pathogenic fungi were isolated, but none of the suspect symptoms gave rise to Itersonilia colonies.

Table 7: Mean % of plant parts with black or brown lesions, July 2009

Sample	Mean plant number sampled	% cotyledons	% petioles	% leaf tips	% crowns	% roots
E15024	50.0	20.0	0.7	0.0	1.3	0.0
E15232	50.0	15.3	0.0	0.7	0.0	0.0
E16042	50.0	16.7	0.0	2.0	2.0	0.7
E14956	43.3	16.9	0.0	2.3	0.0	0.0
E15237	43.3	22.3	0.0	0.0	0.0	0.0
E15011	40.0	20.8	0.0	2.5	1.7	0.0
E15374	50.0	22.0	0.7	0.7	0.0	0.0
E14600	50.0	20.0	0.0	1.3	0.0	0.7
E15375	50.0	12.7	0.0	3.3	1.3	0.7
E15171	33.3	5.0	1.0	1.0	1.0	0.0
E15470	50.0	18.7	0.0	2.7	0.7	0.0
E15249	50.0	14.7	0.7	0.0	0.0	0.7
E15373	50.0	16.0	1.3	3.3	1.3	0.0
E15410	50.0	17.3	0.0	3.3	0.7	0.0
E15339	50.0	18.0	0.7	0.7	0.0	1.3
E15201	50.0	21.3	0.7	1.3	0.0	2.0
1	26.7	27.5	0.0	2.5	0.0	0.0
2	26.7	28.8	1.2	3.7	2.5	0.0
3	26.7	22.5	1.2	3.6	1.2	0.0
2008 re-test	43.3	26.1	0.0	1.5	0.8	0.0

At the October sampling time, black crowns and shoulders were observed. Crown symptoms were either solid black areas, or smaller black streaks. Shoulder symptoms were more diffuse blackened areas. The % of each symptom type is shown in Table 8, together with the recovery of Itersonilia from crown symptoms. No Itersonilia was recovered from shoulder symptoms.

Table 8: Mean % crown and shoulder symptoms, and % of roots with Itersonilia, October sampling

Sample	Mean plant number sampled	% black crowns only	% black crowns and shoulders	% of total roots with Itersonilia
E15024	30	1.1	1.1	0
E15232	30	1.1	0.0	0
E16042	30	0.0	0.0	0
E14956	30	1.1	1.1	0
E15237	30	0.0	0.0	0
E15011	30	0.0	0.0	0
E15374	30	3.3	0.0	0
E14600	30	3.3	0.0	0
E15375	30	1.1	0.0	1.1
E15171	30	0.0	0.0	0
E15470	30	1.1	0.0	0
E15249	30	0.0	0.0	0
E15373	30	2.2	3.3	0
E15410	30	3.3	0.0	0
E15339	30	2.2	2.2	1.1
E15201	30	2.2	0.0	0
1	20	0.0	0.0	0
2	20	0.0	0.0	0
3	20	0.0	0.0	0
2008 re-test	30	3.3	1.1	1.1

At the final sampling in January, a relatively high proportion of roots showed black and brown areas on crowns and shoulders. Itersonilia was recovered from both, though not all infected roots gave rise to colonies. (Table 9)

Table 9: Mean % roots with black crown and shoulder symptoms and % of total roots with Itersonilia, January 2010

Sample	Mean plant number sampled	% black crowns	% black crowns and shoulders	% of total roots with Itersonilia
E15024	136.0	14.0	11.8	1.5
E15232	157.3	10.8	7.0	1.5
E16042	103.7	8.4	6.8	0.3
E14956	64.3	10.9	9.3	0.0
E15237	89.0	14.6	9.4	1.2
E15011	130.7	9.4	7.1	3.6
E15374	158.3	4.4	3.6	0.0
E14600	150.3	15.5	12.2	1.3
E15375	167.3	9.8	8.4	0.6
E15171	141.3	1.4	1.2	0.0
E15470	197.3	6.4	5.6	0.5
E15249	114.3	13.4	9.3	0.9
E15373	96.3	15.6	12.5	2.4
E15410	127.3	5.0	3.1	0.3
E15339	183.7	17.8	12.5	3.6
E15201	193.0	18.0	13.6	3.3
1	59.0	4.5	3.4	0.0
2	82.7	7.7	2.8	0.4
3	88.3	10.9	10.2	4.1
2008 re-test	43.3	25.4	15.4	2.3

Foliar leaf spots which were confirmed as Itersonilia became visible in November 2009. There were significant differences in severity between samples (Table 10). Typical leaf spotting is shown in Fig 9.

Table 10: Severity of Itersonilia leaf spotting (1-5), November 11th

Sample	Itersonilia severity 1-5*
E15024	3.0
E15232	3.7
E16042	3.7
E14956	2.3
E15237	2.3
E15011	1.3
E15374	1.0
E14600	1.7
E15375	1.3
E15171	2.0
E15470	1.3
E15249	1.0
E15373	1.3
E15410	1.0
E15339	1.3
E15201	1.0
1	1.0
2	1.3
3	1.3
2008 re-test	1.0
lsd (p=0.05)	0.92

*1= isolated spots only; 2= few spots in one area; 3 = spots present in more than one area; 4 many spots in some areas; 5 many spots over whole plot

Fig 9 Itersonilia leaf spot symptoms

There were poor correlations between the levels of seed infection and % of roots with black crowns in November (r=0.07) and between seed infection and recovery of Itersonilia from black crowns (r= - 0.06). The same outcome was seen in the January sampling (r = -0.47 and r = - 0.29 respectively). There was also no positive significant relationship between foliar Itersonilia and infection of the seed sample (r = 0.26)

c) Carrots 2008

Low levels of *Alternaria radicina* were recorded in seed tests of the 2008 carrot samples. No other pathogenic fungi were recorded. All were commercial seed lots; two were obtained as treated (T) or untreated (UT) seed (Table 12).

Table 12: % infection with *A. radicina*, 2008 carrot seed samples

Sample	*% A. radicina*
Grace (UT)	0
Grace (T)	0.5
ULYSSES (UT)	0
ULYSSES (T)	0
BOLTEX	0
ELEGANCE	0
ROMANCE	0
BONFIRE	0.5
COREO	0
VAC 48	0
VAC 50	0
MAESTRO	0
ESKIMO	0
Norwich	0.5
NAIROBI	0
NERAC	0
MIAMI	0
BASTIA	0
STANFORD RZ	0
CARRAZZO	0
PRIMO	0

Plants in field plots were harvested and examined for any suspect symptoms of pathogenic fungi. 30 juvenile plants were lifted in July, 50 roots in November and 50 roots in February. A small % of plants on each occasion showed varying symptoms such as leaf browning, petiole and crown blackening and sections of tissue were plated. None gave rise to any pathogenic fungi or bacteria except for one *Alternaria radicina* colony from the Boltex sample in July.

d) carrots 2009

Only two samples in 2009 were infected at low levels with pathogenic Alternaria species. (Table 13). No Cercospora was found. Only two samples were tested for the presence of *Xanthomonas hortorum*, Nairobi and Miami and both were negative.

Table 13: Incidence of Alternaria species in carrot seed samples, 2009

Sample	*% Alternaria dauci*	*% Alternaria radicina*
Nairobi	0	0
Miami	0	0
UK-09-1	0	0
UK-09-2	0	0
UK-09-3	0	0
Vac 48	0.5	1
Vac 50	0	1
8520	0	0
Elegance	0	0
Propeel	0	0

50 plants were sampled in July, and a further 30 in November. Levels of suspect symptoms were generally low (data not shown), and only 1 colony of *Alternaria dauci* was recovered from the base of a petiole in sample 8520. There was no recovery of either Alternaria species from any plant part of Vac 48 or Vac 50, the two samples where some seed infection was recorded. Given the very low incidence of disease symptoms on foliage and roots in November, a further sampling in January 2010 was abandoned.

Discussion

a) parsnips

In both years, seed samples were obtained which were significantly infected with *Itersonilia pastinacae*, but levels of other potential pathogenic organisms were very low or nil. Some seed samples with high Itersonilia had been supplied to growers, but others were obtained from seed companies for the purpose of the project. Possible symptoms of parsnip canker from field grown plants were very infrequent at the early sampling dates in both years, 53 and 59 days after sowing in 2008 and 2009 respectively. When suspect tissues were plated, the recovery of Itersonilia was low in 2008, and nil in 2009. There was no indication that the incidence of Itersonilia in July 2008 was related to the infection level on seed samples.

Despite the limited evidence of early establishment of Itersonilia on juvenile plants, canker symptoms developed by the autumn harvest in both years and incidence increased at the final January harvest. Not all canker symptoms gave rise to Itersonilia. Black cankers tended to give rise to more Itersonilia than brown cankers. A wide range of generally non-pathogenic fungi and bacteria were isolated, with only very occasional Phoma spp colonies (data not shown) which were tentatively identified as pathogenic *Phoma complanata*. Failure to isolate Itersonilia could be due its absence as a causal agent of the symptom, or suppression in culture by saprophytes. However, there was no consistent positive relationship between either the numbers of black cankers or black streak/stripe symptoms and incidence of Itersonilia on seed, or the recovery of Itersonilia from cankers and its incidence on seed, in either year.

In a small scale experiment, inter-plot interference, and movement of discharged ballistospores from one plot to another, is likely to occur, and thus cankers associated with Itersonilia may form on plant samples where seed infection rates were low or zero. However, in plants where seed infection rates were high, it might be expected that canker levels either early or late in the season would also be consistently higher. This was not the case. A total of five samples had around 50% infection, and a further five had around 20% infection on seed. None of these seed lots had consistently higher levels of cankers than those with lower levels of seed infection. In 2008, the highest level of infection (48%) had the lowest % of healthy roots overall at the autumn harvest, but this outcome was not repeated with samples having around 50% infection in 2009.

The results suggest that there is no proportional relationship between infected seed levels and numbers of cankers developing in a crop. However, infected seed is undoubtedly important in introducing disease to previously un-infected land. The trial ground at NIAB has not been used for parsnip trialling for at least 10 years, and is well isolated from major commercial parsnip production. Thus the disease observed on roots is highly likely to have arisen from infected seed. This is in agreement with results reported by Channon (1963), where a parsnip sample with 77% seed infection resulted in 6.5% seedlings infected, though final levels of canker in the mature crop were not evaluated, and only one seed lot was investigated. Spread within a crop from seedling sources probably occurs by means of ballistospores infecting foliage, or landing on exposed crowns and causing direct infection. The extent of spread would depend on many factors, including weather and variety susceptibility. Infected parsnip debris disintegrating in soils then produces resting spores (chlamydospores) which persist and cause infection in following crops.

In commercial production, seed may be a relatively minor source of infection compared to soil-borne resting spores. Given the lack of relationship between seed infection levels and root cankers, a relatively high threshold in seed lots should be acceptable. Though 50% infected seed lots produced no more cankers than 20% lots in this work, a lower threshold might be set, since this is likely to be achievable in most years. 20% infection is suggested as a safe threshold based on the outcomes of this work, but with the proviso that a nil result is preferable to avoid introduction into disease free areas.

Many factors may affect the transmission of a seed-borne fungus to a developing plant, including the extent of colonisation on an individual seed, the vigour of the organism, environmental condition, plant emergence factors, variety resistance, and soil conditions. These factors will govern the inoculum potential of a seed-borne pathogen, and thus it's ability to cause disease. Some seed-borne organisms have the capacity to infect plants efficiently at a very low inoculum level. In the case of Itersonilia though, it appears that a seed-borne infection has a relatively poor disease causing capacity.

One seed sample with just over 20% infection from 2008 was re-tested in 2009. The seed infection had decreased to 1% after 12 months. Decreases in viability have been observed with other samples submitted separately from this project, but data are presented below to illustrate the reductions which might be expected (Table 14). Itersonilia infection may thus decline over time in a period which could be acceptable for commercial seed production, and could offer a means of reducing seed infection without compromising overall quality.

Table 14: Decline in Itersonilia in commercial seed samples over 10 months

Sample	% infection 2007	% infection 2008
A	31	5
B	80	46
C	81	23
D	71	1
E	73	3
F	77	3

b) carrots

Despite the original concerns that seed was a significant source of Alternaria infection in carrots, none of the total of 57 commercial samples examined over three years had high levels of either pathogenic Alternaria species. It was not possible to find conventional seed samples with high levels of Alternaria infection, or other pathogens, even for experimental purposes, during the years of this project. The highest level of *Alternaria dauci* observed, at 3%, in the first year of the project, did not give rise to any observable disease in the field samples taken, either on foliage or roots, despite the absence of fungicides and irrigation to maximise the opportunities for seed to plant transmission. A threshold of 5% infection is thus suggested as acceptable for Alternaria species.

Other, Defra funded, work at NIAB with organic carrot seed has shown that very high levels (75%) of *Alternaria dauci* on resulted in seedling death, severe foliar disease on remaining plants, and small stunted roots. Interestingly, infection levels of the same order of magnitude of Itersonilia, i.e. around 50%, in the parsnip experiments here did not cause seedling death or severe foliar disease and high canker incidence. Alternaria species on carrot seed thus appear to be more aggressive pathogens than Itersonilia on parsnip.

Conclusions

The health status of carrot seed samples examined in this project was high, and even where low levels of Alternaria species were seen, there was no recordable disease observed on carrot foliage or roots in field plots grown from the samples. In parsnip seed samples, levels of *I. pastinacae* were sometimes very high, though no other pathogens were seen at significant levels. However, there was no indication that canker levels on roots were greater in field plots grown from the samples with high seed infection, and a relatively high threshold on seed is likely to be safe in commercial production

Technology transfer

- Outcome of first year experiments summarised and reported to BCGA R&D group, 2007
- Presentation of 2007 and 2008 results to HDC – BCGA Conference, Stockbridge House, March 2009
- Article in HDC News, October 2008
- Article in HDC News, submitted for May 2010
- Project Summary in HDC Vegetable Review, Spring 2010

References

The following references were used in the short literature survey carried out for the first year report and are repeated here for convenience

Channon A G 1963, Studies on parsnip canker. Annals of Applied Biology **51** p1-15
Compendium of Umbelliferous Crop Diseases, 2002. American Phytopathological Society, APS press.
Farrar JJ, Pryor BM, and Davis R M 2004, Alternaria diseases of carrot. Plant Disease **88** p 776 -784
Pryor B M and Gilbertson RL. 2001, A PCR-based Assay for Detection of *Alternaria radicina* on carrot seed Plant Disease, **85** p 18-23
Umesh KC, David RM, and Gilbertson RL 1998, Seed Contamination Thresholds for Development of Carrot Bacterial Blight caused by *Xanthomonas campestris* pv. *carotae* Plant Disease **82** p 1271-1275

