Project title:	Carrot cavity spot - the effects of non-carrot crops on levels of relevant *Pythium* spp. in the soil
Project number:	CP 46
Project leader:	Dr D. J. Barbara Warwick HRI, University of Warwick, Wellesbourne, Warwickshire, CVB35 9EF
Report:	Final report, June 2010. [funding finished 31/12/2009 but this report was delayed until the student had submitted her thesis]
Previous report:	Second Annual report, December 2008
Key staff:	Anne Kretzschmar (PhD Student) Dez. Barbara (Research Leader)
Location of project:	Warwick HRI, Wellesbourne, Warwickshire
Project coordinator:	Mr J Kenyon, Huntapac Produce, Marsh Farm, Marsh Road, Hesketh Bank, Preston PR4 6XT
Date project commenced:	1st January 2007
Date project completed (or expected completion date):	31st December 2009 [when funding was completed; PhD thesis submitted June 2010]
Key words:	Carrot, disease, cavity spot, rotation, soil

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.

D J Barbara
Research Leader
Warwick HRI

Signature ..... Date .....

Report authorised by:

Prof B Thomas
Head of Department
W-HRI

Signature ..... Date .....

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GROWER SUMMARY

Headlines

- Pythium violae has a broad host range amongst both wild (weed) and cultivated species which means that long rotations out of carrots will not ensure freedom from cavity spot.

- Pre-carrot rotations can affect levels of cavity spot disease (potatoes reduced cavity spot by 65 – 70% in two out of three trials).

- "Companion" planting can affect the levels of cavity spot disease, barley was one of the two most effective "companions" - this crop is already used as a nurse crop by some growers without unacceptable losses and further studies on relative planting times may produce a commercially acceptable system.

Background and expected deliverables

Carrots are a major crop in the UK (marketed value £125M-£160M from 2003 - 2006 (Defra: Basic Horticultural Statistics) and the majority of growers consider cavity spot to be their most important crop protection problem. It has been thought that in the UK, cavity spot has been caused by one of two slow growing *Pythium* species. *Pythium violae* seems to be the more widespread of these but *P. sulcatum* also occurs on carrots. Recent work carried out in Norway has cast doubt on a single species being a cause of the disease.; this work has implicated five distinct *Pythium* 'species', including *P. violae* and *P. sulcatum*. It is not clear whether all five of these are important, or even occur, in the UK. At the time this studentship was applied for, the association of the different species of *Pythium* with cavity spot was being investigated and it now appears that *P. violae* is the dominant pathogen in the UK. All trials reported here were carried out in Cottage Field at Warwick-HRI where *P. violae* is predominant.

Disease control is still very problematic despite cavity spot having been the subject of much, largely industry-funded, research over the last few years. Disease avoidance (using only un-infested fields), pathogen elimination (by soil treatments) and early harvesting (before significant damage occurs to roots) are all strategies which have been recommended to reduce the effects of the disease but their application has been limited by several factors. One of these, *viz.* a poor understanding of the dynamics of the relevant pathogens in the soil, is currently being addressed at Warwick HRI in a Defra funded project. As part of this an

effective quantitative test to determine pathogen levels in soil and tissues was developed as this project began.

The main purpose of this studentship was to investigate possible sustainable approaches to the control of cavity spot. These were:

- "Companion planting" - where a second species of plant is grown alongside the crop to provide pest and disease control. At the time of starting this project, there was a small amount of evidence that this was possible but this needed confirming and extending.

- Pre-crop rotations - There is evidence that P. sulcatum can be controlled by a pre-crop rotation of Brussels sprouts (in Australia) and UK growers generally think that long-rotations out of carrots will eliminate or reduce cavity spot caused by P. violae.

It was thought unlikely that commercially acceptable systems would emerge after this three year project but it was hoped that strong reductions in disease of at least 50% would be achievable, by one or both approaches.

Summary of the project and main conclusions

Distribution of disease and inoculum in the soil

The two main parts of the project involved field trials throughout the life of the project. It is known that most soil-borne diseases are not evenly spread in the soil and this was taken into account when designing the field trials. Little published information on heterogeneity in the soil was available at the time when the trials were being designed. Some experience was available at W-HRI from previous projects but this had generally been used to design relatively simple trials with large plot sizes. This project used more complicated designs and therefore it was necessary to use smaller plot and sample sizes to make the trials manageable. The trials were designed based on previous experience but to test whether these designs were adequate, the distributions of disease and fungus across trials and within rows were examined.

There was strong evidence of non-random distribution of disease within individual rows and this was thought to be the reason that levels of disease did not always alter smoothly between sampling dates. *P. violae* was similarly unevenly distributed in rows. Quantities of disease also varied noticeably between individual plots within treatments but there was little evidence for variation at the scale of whole trials.

It was therefore concluded that the trial designs were adequate for looking at large treatment effects but not at small ones. As it was assumed that at least 50% reduction in disease

would be necessary to have commercial relevance, the trial designs were judged in retrospect to be fit for purpose.

Host range of P. violae

Previous projects have shown that *P. violae* is the predominant cavity spot pathogen in the UK. It has long been known that *P. violae* has a wider host range than *P. sulcatum*. However, it was felt that this aspect was worthy of re-evaluation using more modern techniques.

All the crop species used in the rotation and "companion plant" trials were tested for natural infection of the roots along with most of the weeds occurring in Cottage Field (the site of the field trials at W-HRI). Ryegrass and sugar beet were the only two crop species (of 11 tested) which were not at some stage found to support growth of the fungus. At least one plant of all the 15 weeds species tested was found to support *P. violae*. Both crop and weed species varied in the proportion of plants infected and levels of fungus supported but this should be interpreted with care as some of the variation would have been due to heterogeneity in the inoculum. Black nightshade, prickly sow-thistle and knot-grass were the "best" weed hosts.

In pot inoculation experiments all the weeds and crop species tested were infected, generally more frequently than in natural infections. This might be due to the higher inoculum concentration in pots (and more even distribution) or it might be due to a lack of a competing micro-biota in the pasteurised potting soil used. Most likely, both these factors had some effect.

These results emphasise that growth and survival of *P. violae* is not exclusively supported by carrots. This broad host range means it is unlikely that soil after long-rotations out of carrots (or even "virgin" land) will be free of *P. violae*.

Pre-carrot Crops

The effects of different pre-carrot alternate crops were tested in three trials over the three year project. Trials were fully replicated small plots with two two-year trials (alternate crop - carrots) and a three-year trial (alternate crop - alternate crop - carrots). Carrot crops were assessed for disease, levels of fungal growth and relative yield. All trials followed several seasons of cereals (primarily rye) and eight alternate crops were tested. In 2007 and 2009 trickle irrigation was used but in 2008 overhead irrigation. Effects were generally stronger in the first two-year trial and the three-year trial but less good in the second two-year trial during which the alternate crop was grown with overhead irrigation.

Forage rape increased disease in the following carrot crops in all three trials but this was not statistically significant. Barley, sugar beet and wheat gave inconsistent effects whilst clover,

clover/grass mix and broccoli reduced disease by small to moderate amounts in all trials. Potato was the most effective crop in reducing cavity spot – by 65-75% in two of the trials (but only 20% in the third). The different crops had no effect on total yield.
It seems potato could be used as a specific pre-carrot crop to reduce cavity spot disease (but it would be unpopular with growers due to the difficulty of controlling volunteers). These trials established the principle that pre-carrot crops can affect disease levels but whether other crops which are both effective at reducing disease while being more acceptable to growers can be identified will require further work.

"Companion planting"

Three fully replicated one-year small plot trials were used to look at the possibility that "companion planting" (i.e. planting other species with the carrots) would be effective in reducing disease. In the first year 11 species were tested and in following years the range was reduced but timings varied to try to reduce competition with the carrot crop.

In the first trial (2007) five crops reduced disease significantly whilst two (clover and clover/grass mixture) increased it. The two best species reduced disease by over 90% but also greatly reduced yield. In this trial the "companions" were planted at the same time as the carrots and left for the season.

In the second trial the three species most effective in reducing yield were again used, along with clover and a new "companion" *Tagetes* spp. In this trial the "companions" were planted at the same time as the carrots or six weeks later. Late-sown "companions" had no effect on disease whilst four of the early-sown ones reduced disease by more than 50%. Again clover somewhat increased disease. As the late-sown "companions" had no effect on disease, this suggests that the disease needs to be controlled early on in the crop cycle. The early-sown "companions" again reduced yield unacceptably.

In the final trial, three "companions" (barley, wheat, Phacelia) were sown six weeks before the carrots were drilled and removed either at the time of carrot drilling or six weeks later. Disease was reduced by around 95% by the late-removed "companions" but was unaffected by the early-removed ones. Even this short period with the "companions" present meant that competition reduced root weight by approximately 50%. This result emphasised that the outcome of the epidemic is determined sometime during the first six weeks of the crop.

These trials demonstrated that "companion" planting can reduce cavity spot disease by large amounts but showed that the major problem is yield loss due to competition. Barley, the most effective "companion", is already used by some growers as an early "nurse" crop and further manipulation of the relative sowing dates may allow a commercially acceptable system to be developed.

Mechanisms

The possible mechanisms by which these effects on disease occur are discussed in the thesis. Suffice it to say here that the results from this project when taken together with results from other projects, suggest that cavity spot disease is modified through effects on competing micro-biota rather than on direct reductions in the amount of *P. violae* found in the soil.

Financial benefits

The trials described here have not yet led to commercially acceptable ways of reducing cavity spot disease and so no financial benefit can be calculated.

Action points for growers

- *P. violae* has a wide host range amongst other crops and weeds and there is little benefit from long rotations out of carrots. On the other hand, very short rotations with carrots should still be avoided as non-cavity spot factors will reduce yield if carrots directly follow carrots. Two years of other crops between carrot crops is probably safe but this has not been tested directly.

- "Companion" planting cannot yet be recommended to growers as the loss of yield is unacceptable. Further work may come up with a commercially acceptable system.

- Most crops in the year or two years before carrots had little effect on disease levels. Forage rape slightly increased disease and probably should be avoided. From a cavity spot view, potatoes in the previous year is a good thing but growers should carefully consider their attitudes to controlling volunteers before deliberately choosing fields which have had this crop.

SCIENCE SECTION

This report is an edited version of the thesis (with some material from earlier reports) submitted by A Kretzschmar to the University of Warwick in June 2010. As the thesis is ca. 400 pages this report represents a significantly shortened version but includes the important results.
The full version of her thesis will be available through the University Library once Ms Kretzschmar has completed her oral exam and been awarded her degree.

Introduction

Cavity spot on carrot was initially considered a physiological disorder caused by waterlogging (Guba *et al.*, 1961) until Lyshol *et al.* (1984) discovered that the incidence of cavity spot can be reduced by application of metalaxyl-containing fungicides. Groom and Perry (1985) successfully induced cavity spot lesions on carrots by inoculating roots with *Pythium violae*. In Canada, Benard and Punja (1995) found several *Pythium* species, with *P. violae* and *P. sulcatum* being most virulent, to be causal agents of cavity spot in carrots. Both species have since been found to cause severe losses in carrot crops due to cavity spot worldwide (Hiltunen and White, 2002).

Cavity spot on carrots, caused by *Pythium sulcatum* or *Pythium violae*, has been reported from Australia (El-Tarabily *et al.*, 1996), Canada (Allain-Boulé *et al.*, 2004), United Kingdom (White, 1986), Norway (Hermansen *et al.*, 2007), France (Montfort and Rouxel, 1988), the Netherlands (Wagenvoort *et al.*, 1989), Israel (Zamski and Peretz, 1995) and the USA (Schrandt *et al.*, 1994). In the United Kingdom *Pythium violae* is reported to be the most common pathogen causing cavity spot (Hiltunen and White, 2002).

Pythium violae Chesters and Hickman is a soil-borne pathogen with a wide host range (Schrandt *et al.*, 1994, Hiltunen and White, 2002). Initially cavity spot lesions are small, sunken and dark. Below an intact layer of periderm infected phloem cells collapse, forming the sunken lesion. At a later stage the periderm splits and the lesion heals by forming layers of suberised cells, or secondary pathogens invade the lesion (Perry and Harrison, 1979). The infection process has been studied and described in detail (Zamski and Peretz, 1995; 1996; Campion *et al.*, 1997; 1998; Cooper *et al.*, 2004).

Inoculum of *Pythium violae* is assumed to remain in the soil for many years (Hiltunen and White, 2002). As a result carrots are grown in wide rotations or in fields where no carrots have previously been grown (Hiltunen and White, 2002). Increasingly intensive production of carrots makes it more difficult to find suitable fields which fulfill these conditions. In carrot fields cavity spot is sometimes controlled by the application of metalaxyl-based anti-oomycete fungicides during the growing season (White 1988; Hiltunen and White, 2002; Suffert *et al.* 2008). Davison and McKay (1999) and Kenny *et al.* (2001) reported that after

repeated application metalaxyl is broken down rapidly by soil microbes, leading to a reduced effectiveness. White *et al.* (1988) found some *P. sulcatum* isolates to be tolerant to metalaxyl. Therefore it is necessary to find alternative ways of controlling cavity spot disease in carrots and the inoculum of *Pythium violae* in soil. Good soil aeration provided by growing carrots on ridges and the application of soil conditioner was found to reduce cavity spot in carrots (Jacobsohn, 1984). El Tarabily *et al.* (1996) controlled cavity spot by applying lime to the soil, thereby raising soil-pH. White reported little or no cavity spot disease in fields with a soil-pH higher than 8.0. Other authors did not find a significant influence of pH on incidence of cavity spot (Vivoda *et al.*, 1991). High soil moisture is considered favourable for the growth of *P. violae* and development of cavity spot (Jacobsohn, 1984; Vivoda *et al.*, 1991; Hiltunen and White, 2002), epidemics were observed after periods of flooding or heavy rainfall (Vivoda *et al.*, 1991), whereas the incidence of cavity spot can remain low in dry summers (Suffert *et al.*, 2008).

Theunissen and Schelling (2000) described companion planting as one possible mean of control of cavity spot and carrot root fly. They found significant reduction of cavity spot disease in carrots undersown with subterranean clover (*Trifolium subterraneum*). However, due to competition the yield of carrots was also greatly reduced. Barbara (pers. comm.) found a significant reduction of cavity spot (*P. violae*) in carrots grown in beds with wheat and beetroot. Field trials in Australia (Davison and McKay, 2003) showed that broccoli planted in a rotation before carrots reduced the amount of cavity spot in carrots, which was caused by *Pythium sulcatum*. Jacobsohn *et al.* (1984) did not find any influence of preceding crops on cavity spot. *P. violae* is assumed to have a wider host range than *P. sulcatum* (Hiltunen and White, 2002). Schrandt *et al.* (1994) found several crops to be hosts of *P. violae*, not all of them showing symptoms of disease. More research on the effects of non-carrot species within rotations and of companion planting for controlling cavity spot in carrots is needed in order to develop practical systems of control. Within the project these mechanisms were examined in three rotation trials and three companion plant trials.

It is also not fully understood in what forms *P. violae* persists in soil, for how long and what role alternative hosts play in maintaining inoculum levels (Hiltunen and White, 2002). Lesions of carrots and whole carrots infected with *Pythium violae* were found to remain infectious to healthy carrots in pot trials (Suffert and Montfort, 2007). That indicates that it is possible that mycelium on plant debris or infected plants could act as a source of inoculum. *Pythium violae* is not pathogenic to all of its hosts (Schrandt *et al.*, 1994) so non-carrot crops and weeds may be a source of inoculum even if *P. violae* does not cause disease to them. All non-carrot plants used in field trials within the project and some common arable weeds were tested to see whether they are host plants of *P. violae*, and which of these species could maintain and renew its inoculum.

Pythium violae produces oospores (van der Plaats-Niterink, 1983), which are thought to be an important resting structure. It is not known for how long they stay viable in soil and what induces their germination. *Pythium sulcatum* was found to remain viable in soil for at least 21 months (Hiltunen and White, 2002), oospores of other *Pythium* species are thought to persist for more than 12 years (van der Plaats-Niterink, 1983). Environmental conditions affecting the oospores of *P. violae* are poorly known and in preliminary experiments the conditions in which oospores of *Pythium violae* are produced and the influence of external factors on their survival and germination were examined.

It has also been shown that *P. violae* can proliferate rapidly in soil during a season and cause a cavity spot epidemic if the conditions in soil are favourable during the growing season (Phelps *et al.*, 1991; Suffert *et al., 2008*; Suffert and Montfort, 2008). Even though relatively reliable PCR-based testing systems have been developed (Klemsdal *et al.*, 2008; D Barbara, unpublished), time and severity of a cavity spot epidemic cannot be predicted from soil inoculum levels before planting (Barbara, unpublished; Suffert, 2007) That shows that it is important to apply measures of control *P. violae* before and during a carrot growing season.

Aspects of cavity spot to be investigated

Two main ideas were to be studied.

1) That non-carrot plants grown before and during the carrot growing season influence the growth and development of *Pythium violae* and hence of cavity spot disease. The potential practical outcomes of this are (i) a clearer understanding of the effects of pre-carrot crops in the rotation so that growers may look to minimize disease by using fields in which crops which reduce disease have been grown (and avoid fields where crops which increase disease have been grown) and (ii) to study whether “companion plants” can reduce the disease.
2) That non-carrot host plants can play an important role in maintaining levels of inoculum over many years in the absence of carrots by allowing *P. violae* to proliferate and form new resting structures.

Specific Objectives

- To compare several rotations to test whether they influence the amount of inoculum in soil, and the growth of the fungus and the severity of cavity spot disease in the following carrot crop. Ideally rotations which reduce cavity spot in carrots by at least 50% would be found and could be recommended to growers.
- To examine different companion planting systems as a mean of cavity spot control within a carrot season. The aim was to find suitable companion plants and planting

designs which reduce cavity spot in carrots with the least possible carrot yield reduction through competition.

- To test several crop and wild plant species for their role in maintenance and build up of inoculum. Plants grown in naturally infested fields and plants inoculated in pot trials were to be tested for their role of potential hosts to *P. violae*.

Materials and methods

Field trials

All field trials were set up in Cottage Field at WHRI Wellesbourne, UK. The soil at Cottage Field was described by Whitfield (1974) as a typical Brown earth, further classified as shallow Wick soil (wQ_2): A-horizon: very friable sandy loam, weakly developed medium and coarse block structure, abundant medium and large pores: B-horizon: slightly stony sandy loam, structure less, stones mainly rounded pebbles or flints: C-horizon: stony loamy sand, fossils, shells, pebbles and flints, limestone fragments, structure less, highly calcareous.

Assessment of cavity spot symptoms on carrots

Carrots were washed by hand after the surface soil was sampled and cavity spot lesions were counted on each root. Only lesions which matched the description of cavity spot damage in the literature (Hiltunen and White, 2002) were included. Only lesions with a diameter larger than 2 mm but smaller than 2 cm were counted.

Soil Sampling

Soil from fallow beds or open spaces between rows of vegetation was taken and referred to as open soil. Samples were taken from a depth of approximately 15 cm below the surface. Bulk samples of five cores per plot were taken from each plot and air dried on a bench. Any visible roots, plant debris and stones were removed. The samples were homogenized by rolling before soil DNA extraction. For soil off roots, harvested roots were spread out on a bench and left to dry overnight then the dried soil on the surface brushed off by hand and collected. Samples were homogenized by crushing with a roller before DNA extraction. Samples were stored in individual polythene bags or tubes and kept at 5°C in the dark for up to 14 days before DNA extraction.

DNA extraction

DNA was extracted from soil samples using the MoBio PowerSoil DNA Isolation Kit followed by a DNA purification through PVP (AppliChem GmbH, Germany) as described in Klemsdal *et al.* (2008). DNA was extracted from 0.25 g of homogenised soil sample according to the

manufacturer's protocol unless stated otherwise. Initial disruption used a FastPrep cell disruptor for 30 s at a speed of 5.5 m/s.

Washed plant roots of freshly harvested were freeze-dried before DNA was extracted using the DNeasy Plant mini kit (Qiagen) protocol, modified by Morton (pers. comm.).

For fungal DNA, aerial mycelium taken from the surface of plates was extracted by a squash blot on Nytran N membranes (Schleicher & Schuell BioScience GmbH, Germany) (Langridge *et al.* 1991) followed by a PVP clean-up (Klemsdal *et al.* 2008). DNA samples were stored at -20^{o}C in the dark.

PCR

End-point PCR used standard protocols, with appropriate controls, and primers as in Table 1. When required, sequencing of amplicons used standard procedures. A SYBR-green based quantitative PCR protocol developed by Barbara (as part of a Defra-funded project) with primers PVIOLf and PVIOLr (Table 1) was used to quantify *P. violae* DNA in samples. The use of the other primers listed here is not described in detail in this report. Detailed discussion of the reliability and meaning of this quantification is given in the thesis but is beyond the scope of this report.

Table 1: Primer pairs used for quantitative and end-point PCR

Species	Primer	Sequence (5' to 3')
Pythium intermedium	PINTER f[1]	ATGCAGAGGCTGAACGAA
	PINTER r[1]	CTGTATTCATAGCCGAAACGA
Pythium sulcatum	PSULC f[1]	GCCGCTTTATTGTGGTCT
	PSULC r[1]	TCTTCTTTACCCCACAAGTGA
Pythium violae	PVIOL f[1]	ATGTGTGTGTGCGGGACT
	PVIOL r[1]	CCACTCCCCAAAGAGAGAAGT
	PV1[2]	GTGTGCGGGACTGGCTGAT
	PVIOL r[1]	CCACTCCCCAAAGAGAGAAGT

[1] Klemsdal *et al.* (2008) [2] Wang *et al.* (2003)

Isolation and culture of P. violae.

Isolation, culture and storage of *P. violae* used standard mycological techniques as described in the thesis.

Results

I: Host plants of *Pythium violae* and detection of *P. violae* in tissue and soil samples

Aims of the experiments

This section was aimed at giving a broad insight into the host range of *Pythium violae* amongst wild and cultivated plants, without determining the precise role of each species in the epidemiology of cavity spot or reliably distinguishing between important hosts of *P. violae* and plants which are only occasionally colonized. The crops used within the companion planting trials and the rotation trial (see later sections) were included as well as the majority of the weed species in Cottage Field at W-HRI. It was also to be studied if *P. violae* was pathogenic to all plant species it colonized. The findings of these host plant experiments were expected to help understand results from the rotation trial and the companion trial.

P. violae and non-carrot plants

Over the course of the project, *P. violae* was detected in the roots at least some individual plants of most of the crop and weed species tested (see fig 1 for examples) when grown in Cottage Field, either as part of the trials or, in the case of weeds, growing within plots or in nearby fallow land. The proportions of each plant type testing positive varied greatly from year to year. Ryegrass and sugar beet were the only two crop species not found to support *P. violae* at some stage. At least one plant of all 15 weed species tested was found infected at some point during the project. *Solanum nigrum*, *Sonchus asper* and *Polygonum aviculare* were the species which generally showed the highest proportions of infected plants. In no case were all plants of any given species infected but this probably just reflects the discontinuous distribution of inoculum.

The amounts of DNA detected in samples from individuals within the species varied greatly but amongst the weeds *Solanum nigrum* seemed to support particularly high levels of the fungus.

Elsewhere in this project and in other project, *P. violae* has been frequently found in the soil closely associated with carrot roots. Here *P. violae* was only detected in root surface soil samples from beetroot, marigold, wheat and rape of the crop species (Figure 1). *P. violae* was detected at some stage in root surface soil of all wild plants with the exception of *Taraxacum officinale* and *Veronica persica*.

In these experiments it is difficult to separate the effects of unevenly distributed inoculum from variable sensitivity of the plant types. However it is clear that many species other than carrot can act as host to *P. violae*.

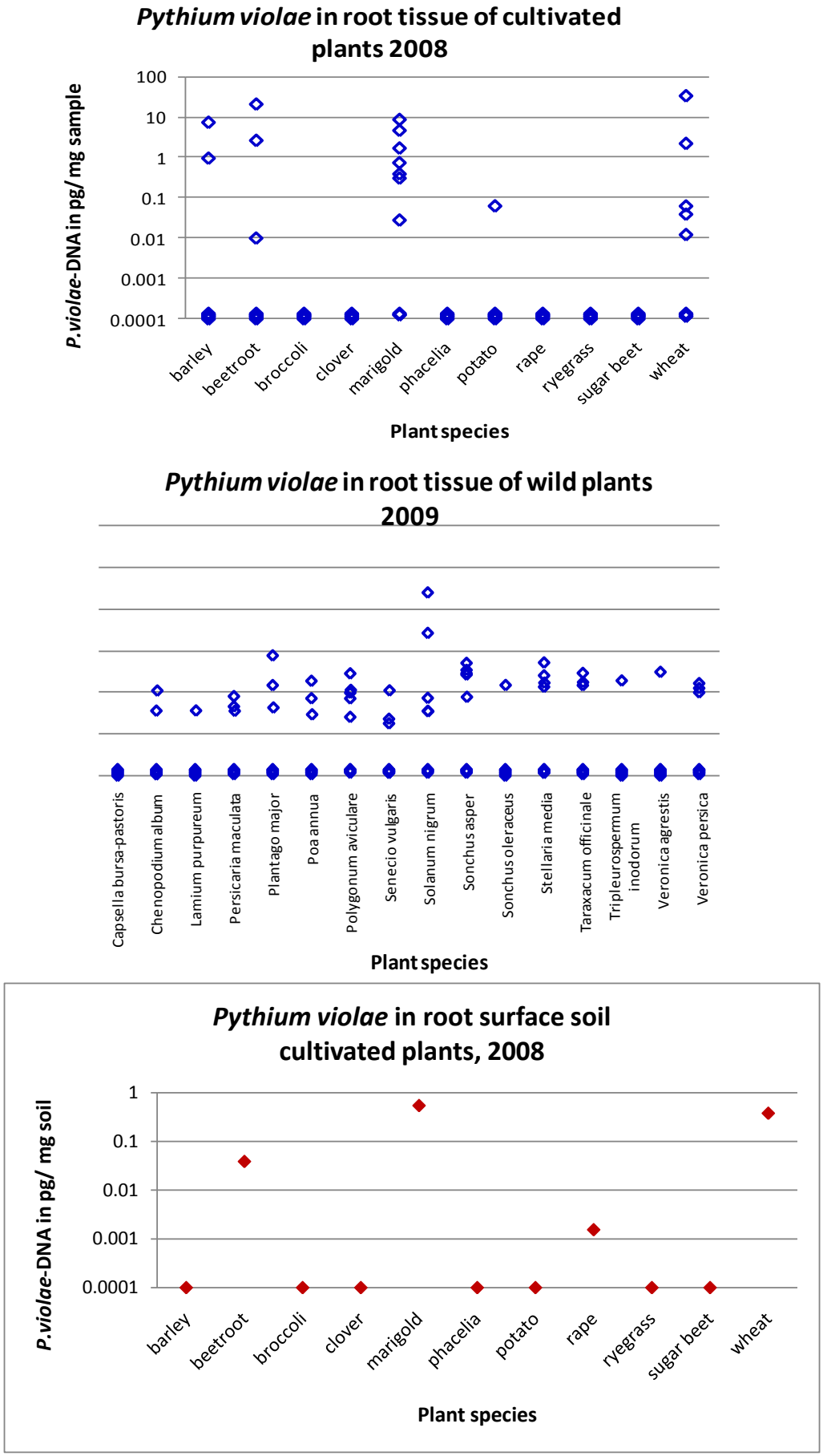

Figure 1: Amounts of DNA of *Pythium violae* in root tissue of cultivated (top) and weed species (middle) and in bulk samples of root surface soil from cultivated plants from Cottage Field (bottom). For tissue 10 root samples per species were analysed. All DNA amounts are given in pg / mg freeze dried sample.

In pot inoculation trials, *P. violae* was detected in at least one root tissue sample of each of 14 crop species (12 plants sampled per species). Most often it was found in beetroot, leek, *Nicotiana clevelandii*, rape, sugar beet and wheat. The amounts of *P. violae* in infected root tissue did not differ greatly between species. In 2008 there were problems with the design of the experiment in that infection was found both in deliberately inoculated pots and also in control pots.

Weed species were only used in pot inoculation trials in one year (2009). *P. violae* was found in roots of all 14 wild plant species tested, except *Senecio vulgaris*. Most often it was found in roots of *Tripleurospermum inodorum*. The highest amounts of *P. violae*-DNA were found in roots of *T. inodorum*, *Veronica agrestis*, *V. persica*, *Lamium purpureum* and *Taraxacum officinale*. *P. violae* DNA was detected in some samples of soil off the roots of all cultivated species at some stage but definitively only in soil off the roots of *Senecio vulgaris, Veronica agrestis, V. persica* and *Poa annua* amongst the weeds.

There was little evidence of pathological effects on any of the plants tested by pot inoculation except on *Nicotiana clevelandii* where plant weights were reduced. This is unlikely to be important commercially to carrot growers as this species is only grown in the UK as a garden ornamental. Various signs of damage and discolouration were seen on the roots of inoculated plants but *P. violae* was as likely to be associated with healthy roots as with the diseased ones.

II: The effects of pre-carrot-crop rotations on cavity spot and growth of *P. violae*

Aims of the experiments.

Many growers assume that crop rotations have an effect on cavity spot, most important being a long interval between carrot crops. However, few data are available on the effects of crop rotations on cavity spot disease or inoculum levels of *Pythium violae* and *Pythium sulcatum*. Published studies suggest that long rotations might be needed to reduce the inoculum of *P. violae* and *P. sulcatum* (Hiltunen and White, 2002). In Australia, cavity spot damage, there caused by *P. sulcatum*, was reduced by planting broccoli in the two years between two carrot crops (Davison and McKay, 2003). However, *P. sulcatum* and *P. violae* are assumed to have different host ranges (Hiltunen and White, 2002), so the effects of rotations on cavity spot disease caused by *P. violae* cannot be inferred from results from trials on *P. sulcatum*. The experiments here were intended to provide data on the effects of various preceding crops on levels of cavity spot and *P. violae* inoculum in replicated trials at W-HRI. The practical aim was to find rotations which reduced cavity spot in subsequent carrot crops by at least 50%.

Experimental Design

The rotation trial consisted of three sets of rotations, each set comprising nine treatments. Two sets of rotations were started in 2007; the first two-year rotations and the three-year rotations. The second set of two-year rotations was started in 2008. All rotation experiments started with rye as a preceding crop in 2006 and 2007 respectively. Rye was followed by nine different crops (Table 2), preceding carrot in the last year. In the three-year rotation set the nine preceding crops were replanted in the same plots over two years, followed by the carrot crop in the last year.

The rotation trial design was similar to a Latin square, with the two factors "rotation" and (preceding) "crop". The rotation was the main factor; the crop was the sub-factor, randomised within each repeat of the rotation. The three rotations were randomised between the nine blocks of the field; the nine preceding crops were randomised within each block. The design is similar to a Sudoku matrix; each rotation appears only once in each row and column of blocks within the field, and the crops appear only once in each row and column of beds within the field (Figures 2 & 3). The field consisted of nine beds, each 1.83 m wide and 28 m long. The space between adjacent beds was approximately 50 cm.

In 2007 the plots were trickle-irrigated with 45 mm per week after the seedlings had established. In 2008 the plots were irrigated overhead throughout the season. In 2009 trickle irrigation was installed after seedlings emergence as described for 2007 because overhead irrigation had proved insufficient in 2008. Fuller details of establishing this trial is available in the full thesis.

Open soil was sampled from each plot after harvests in 2007 and 2008 and before sowing in 2007, 2008 and 2009. A bulk sample of carrot root surface soil from ten carrots per treatment was collected at each harvest.

The first two-year rotation trial was harvested in October 2008. 80 carrots per bed were harvested from the inner two rows of each bed. A bulk sample of carrot surface soil was taken from ten of these carrots per bed. From these samples DNA was extracted and analysed. The carrots were washed and the cavity spot lesions counted. In October 2009 the second two-year rotation and the three-year rotation were sampled as described for 2008 although from two of the three broccoli plots in the second two-year rotation only 33 and 36 carrots instead of 80 carrots were harvested, due to the small fraction of the plots in which broccoli had established in 2008. The statistical analysis used is described in the thesis.

Table 2: Rotation trial; all rotations 2007 – 2009.

Year	**2007**	**2008**	**2009**
Rotation			
1st two-year rotation	barley	carrot	-
	broccoli	carrot	-
	clover	carrot	-
	clover-grass	carrot	-
	potato	carrot	-
	rape	carrot	-
	sugar beet	carrot	-
	wheat	carrot	-
	carrot	carrot	-
2nd two-year rotation	rye	barley	carrot
	rye	broccoli	carrot
	rye	clover	carrot
	rye	clover-grass	carrot
	rye	potato	carrot
	rye	rape	carrot
	rye	sugar beet	carrot
	rye	wheat	carrot
	rye	carrot	carrot
three-year rotation	barley	barley	carrot
	broccoli	broccoli	carrot
	clover	clover	carrot
	clover-grass	clover-grass	carrot
	potato	potato	carrot
	rape	rape	carrot
	sugar beet	sugar beet	carrot
	wheat	wheat	carrot
	carrot	carrot	carrot

The clover-grass mixture was produced by mixing perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) in a weight ratio of approximately 9:1, which equates to a ratio of grain numbers of approx. 5:1.

barley	carrot	clover	broccoli	potato	clover-grass	rye	rye	Rye
broccoli	clover-grass	rape	sugar beet	carrot	wheat	rye	rye	rye
wheat	sugar beet	potato	barley	rape	clover	rye	rye	rye
rye	rye	rye	carrot	sugar beet	potato	broccoli	rape	clover
rye	rye	rye	rape	clover-grass	broccoli	carrot	wheat	barley
rye	rye	rye	wheat	clover	barley	potato	clover-grass	sugar beet
rape	clover	carrot	rye	rye	rye	wheat	potato	broccoli
sugar beet	broccoli	barley	rye	rye	rye	clover	carrot	clover-grass
potato	wheat	clover-grass	rye	rye	rye	sugar beet	barley	rape

2-year rotation, start 2007
3-year rotation, start 2007
2-year rotation, start 2008

Figure 2: Field plan of rotation trial in 2007. All crops were planted from 4 to 10 April 2007.

carrot	carrot	carrot	broccoli	potato	clover-grass	rape	sugar beet	wheat
carrot	carrot	carrot	sugar beet	carrot	wheat	barley	clover	potato
carrot	carrot	carrot	barley	rape	clover	clover-grass	broccoli	carrot
clover-grass	barley	wheat	carrot	carrot	carrot	broccoli	rape	clover
clover	potato	sugar beet	carrot	carrot	carrot	carrot	wheat	barley
carrot	rape	broccoli	carrot	carrot	carrot	potato	clover-grass	sugar beet
rape	clover	carrot	clover-grass	barley	sugar beet	carrot	carrot	carrot
sugar beet	broccoli	barley	potato	wheat	rape	carrot	carrot	carrot
potato	wheat	clover-grass	clover	broccoli	carrot	carrot	carrot	carrot

2-year rotation, start 2007
3-year rotation, start 2007
2-year rotation, start 2008

Figure 3: Field plan of rotation trial in 2008. All crops were planted on 22 and 23 April 2008. In 2009 the beds in carrots for the first 2 year rotation were not used in this experiment and all other beds were drilled with carrots on 14th May.

In the first two-year rotation harvested in 2008, the levels of disease were lower than in the two rotations which were harvested in 2009, probably as a result of using overhead irrigation in 2008. The proportions of diseased carrots did not differ significantly between the second two-year rotation and the three-year rotation, harvested in 2009.

The lowest proportion of diseased carrots was found in rotations with potato as a preceding crop, followed by clover-grass mix and broccoli. The highest proportion of cavity spot disease was found in plots with forage rape, sugar beet, barley and carrots as preceding crops (Table 3). Over the three rotations the difference between rotations with the lowest

and the highest amounts of cavity spot appears to be more pronounced in the three-year rotation set, but the interaction effect between rotation and preceding crop was not statistically significant. The frequency distributions of lesion numbers per diseased carrot did not differ greatly between rotation sets and preceding crops. The geometric means of lesion numbers per carrot varied significantly only between the three sets of rotations. In the first two-year-rotations fewer lesions per carrot were found than in the second set of two-year rotations and the three-year rotations. The average numbers of lesions per root did not vary significantly between carrots following different preceding crops. The highest and the lowest average lesion numbers in each rotation set differed only by approximately one lesion per root (data not shown but given in thesis).

Table 3: Proportion (%) of diseased carrots in two- and three-year rotations with nine preceding crops. 80 carrots were sampled per plot, except for carrots grown after broccoli in the second two-year rotation, (only 33 and 36 carrots were sampled from two plots). Arithmetic means, SEDs and Tukey's LSDs were calculated in ANOVA at $\alpha = 0.05$.

Rotation Preceding crop	1st 2-yr	2nd 2-yr	3-year	measure	rotation	crop	combined
barley	11	54	34	F pr.	< 0.01	< 0.01	0.37
broccoli	11	30	26				
							10.0
carrot	16	35	42	SED	6.0	4.9	(8.5[1])
clover	15	30	37	LSD	14.7	9.9	20.4
clover-grass	10	30	19				
potato	4	28	15	df. res.			48
forage rape	19	50	47				
sugar beet	13	51	38				
wheat	6	44	35				

[1] SED for comparisons within same level of treatment

The average carrot root weight was lower in the first set of two-year rotations than in the second set of two-year rotations and the three-year rotations (again probably due to the use of overhead irrigation in 2008). Carrots grown after carrots were lighter than carrots grown after other crops. This difference was most pronounced in the first two-year rotation and in the three year rotation. The highest root weights were observed in carrots grown after broccoli. The average root weights between the remaining treatments did not vary significantly (Table 4).

Table 4: Average root weight in g in two- and three-year rotations with nine preceding crops. 80 carrots were sampled per plot, except for carrots grown after broccoli in the second two-year rotation (only 33 and 36 carrots sampled from 2 plots). Means, SEDs and Tukey's LSDs were calculated in ANOVA at α = 0.05.

Rotation Preceding crop	1st 2-year	2nd 2-year	3-year	measure	rotation	crop	combined
barley	65	78	83	F pr.	< 0.01	0.02	0.12
broccoli	67	95	77				
carrot	42	77	73	SED	4.7	3.8	7.8 (6.5)
clover	55	85	75	LSD	11.6	7.6	
clover-grass	61	78	80				
potato	55	76	78	df. res.			46
forage rape	54	73	81				
sugar beet	65	75	83				
wheat	67	79	75				

Pythium violae in soil

In open soil from the first two-year rotation, sampled in autumn 2007 until winter 2008, and in samples taken before drilling in spring 2008 little *Pythium violae* was found. In autumn 2007 *P. violae* was detectable in four open soil samples from plots in which carrots, clover and clover-grass mix had been grown in 2007. In spring 2008 *P. violae* was detectable in few plots from all rotations in quantities of around one genome of *P. violae* per mg soil (0.02 - 0.04 pg DNA). The values detected in carrot surface soil were about ten times higher than in open soil before sowing. The variation within the three plots of each crop was higher than the variation between crop treatments. The highest amounts were found on carrots following carrot, the lowest on carrots following wheat.

In the second two-year rotation trial, less than the equivalent of one genome or no *P. violae* was found in most samples from the autumn and spring before the carrot crop. The amounts in root surface soil varied greatly within each treatment and between treatments. The highest amounts were found on carrots grown after barley, the lowest on carrots following carrot or potato.

In open soil from the three-year rotation trial, sampled in autumn 2008 and spring 2009, either no or a maximum of the equivalent of approximately one genome of *P. violae* per mg soil was detected, except in one autumn sample from one plot in which potatoes had been grown in which ten times this amount was detected. The amounts of DNA found in carrot surface soil varied strongly within all treatments. The amounts of *P. violae*-DNA were smallest on carrots following carrots. The highest levels were detected on carrots grown

after wheat, barley and sugar beet (data for this trial given in Figure 4; for data for 2-year trials see thesis).

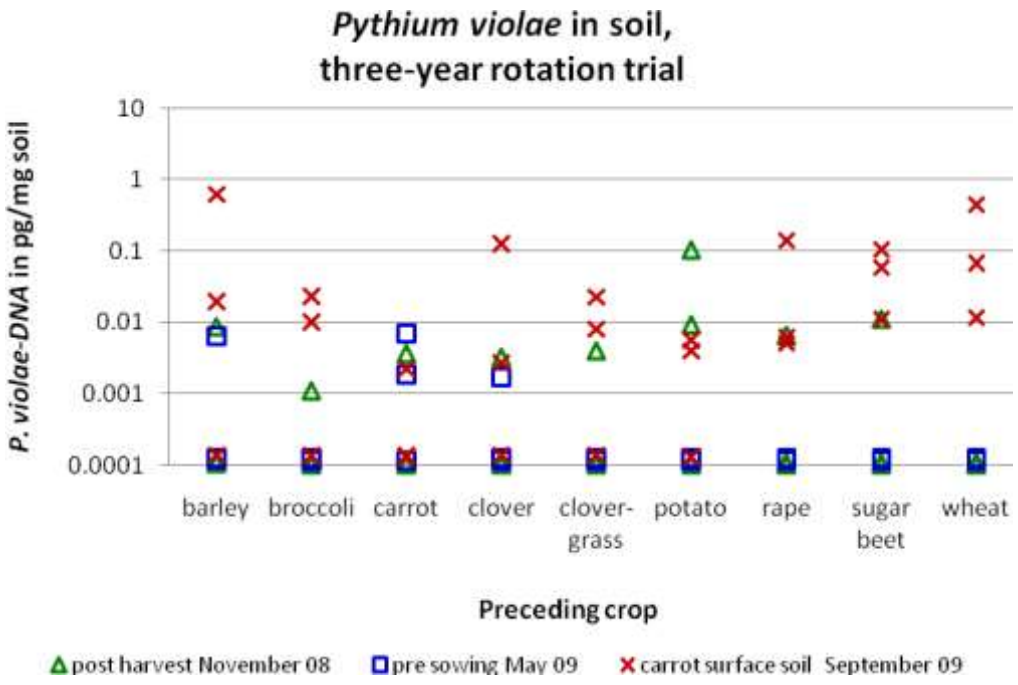

Figure 4: DNA of *Pythium violae* (pg/mg) in open field soil after previous harvest and before sowing, and in carrot surface soil in the three-year rotation 2007 to 2009.

No preceding crop eliminated soil inoculum of *P. violae* and no crop led to an increase in detectable soil inoculum levels compared to a rotation of consecutive carrot crops in all three trials. The differences within each treatment were mostly greater than the differences between treatments.

Correlation between P. violae *in soil and cavity spot*

The amounts of *P. violae*-DNA detected in open soil after removal of the last preceding crop and before sowing, were not significantly correlated with the proportion of carrots with cavity spot. Only the amounts of *P. violae*-DNA in carrot surface soil were weakly correlated with the proportion of diseased carrots. The correlation was strongest in the second two-year rotation and weakest in the three-year-rotation, for which it was not statistically significant (Table 5).

In none of the rotation sets were any statistically significant correlations found between the amounts of *P. violae*-DNA in soil, sampled after removal of the preceding crop, the amounts in soil sampled prior to sowing the carrots in spring, and the amounts of *P. violae*-DNA in carrot root surface soil at harvest (data not shown here).

Table 5: Spearman's rank correlations between proportions of diseased carrots and amounts of *P. violae*-DNA in soil samples. r_s is Spearman's rank correlation coefficient, the p-values are provided for two-sided test at $\alpha = 0.05$.

Rotation	1st 2-year rotation		2nd 2-year rotation		3-year rotation	
	correlation with proportion of diseased carrots					
Soil sample	r_s	p-value	r_s	p-value	r_s	p-value
post harvest 2007	0.27	0.17	-	-	0.38	0.047
pre-sowing 2008	0.22	0.27	- 0.12	0.20	- 0.05	0.80
carrot surface soil 2008	0.43	0.03	-	-	-	-
post harvest 2008	-	-	- 0.12	0.57	- 0.37	0.06
pre-sowing 2009	-	-	0.21	0.30	0.27	0.18
carrot surface soil 2009	-	-	0.62	< 0.01	0.33	0.09
sample size	27					
df.	25					

III: Effects of "companion plants" on cavity spot disease and growth of *P. violae*

Aims of the experiments

There is some published and some unpublished evidence that "companion planting" (or interplanting) can reduce cavity spot disease (Theunissen and Schelling, 2000; Barbara unpublished). The aim of the experiments here was to develop a combination of carrots with other plants which gave more than 50% reduction in cavity spot disease whilst leaving carrot crop yield substantially unaffected.

The reasoning for the initial choice of potential companion plants is given in detail in the thesis.

Trial Design.

The first companion plant trial in 2007 was planted in a column-row α design with four blocks (Figures 6 & 7). Each block comprised a full set of treatments and a carrot control without companion plants. Eleven companion plant species were planted between triple rows of carrot. Greater detail for the design and layout of the trial is given in the thesis. Wheat, barley, leek, clover, clover-grass, grass and *Nicotiana clevelandii* were planted in a distance of approx. 5 cm to both sides of the inner two triple rows of carrots. The outer carrot rows were 20 cm away from the companion rows. This design allowed examination of the effect of the two distances on the growth of the carrots and the development of cavity spot disease

without doubling the number of beds. Beet root, broccoli, Indian mustard and phacelia were expected to compete strongly with the carrots. They were sown between two carrot rows approx. 15 cm away from the rows.

Figure 6: Companion planting trial, May 2007, after emergence of carrots and companion plants.

A bulk sample of soil from the upper 15 cm was taken from each plot before sowing on 23 April 2007 and one month after sowing on 12 June 2007. At every harvest, carrot surface soil from 24 carrots was taken from each plot as a single sample for DNA extraction. The DNA-extracts were tested for *P. violae*, *P. sulcatum* and *P. intermedium* by PCR. Carrots were harvested five times during 2007 at intervals of approximately four weeks. At every date 24 carrots were taken from each plot in sets of six. Carrots from the inner two rows, referred to as "inner carrots", were harvested from all treatments.

At every harvest a bulk sample of at least ten companion plants per plot was taken and root surface soil was collected for DNA extraction as described for carrots. The DNA-samples were tested for *P. violae*, *P. sulcatum* and *P. intermedium*.

Statistical analysis for this and the two later trials is described in the thesis.

ryegrass	Indian mustard	Carrot (no comp.)	barley	beetroot	clover
clover	broccoli	wheat	Carrot (no comp.)	leek	phacelia
barley	phacelia	nicotiana	ryegrass	wheat	clover-grass
leek	beetroot	clover-grass	broccoli	Indian mustard	nicotiana
clover	ryegrass	phacelia	Carrot (no comp.)	barley	wheat
Carrot (no comp.)	broccoli	beetroot	clover	leek	Indian mustard
nicotiana	leek	wheat	clover-grass	broccoli	phacelia
Indian mustard	clover-grass	barley	beet	nicotiana	ryegrass

Figure 7: Field design for companion plant trial in 2007**.**

In 2008, five companion species were used, combined with two times of sowing and a non-companion control. Phacelia, wheat and barley were used because carrots grown in plots with these species showed reduced disease levels in 2007 compared to the controls. Clover was planted because an increased cavity spot incidence in carrots had been observed in beds with clover as a companion. French marigold was included as a new companion species because it has been used as a trap crop against nematodes and shown to be effective against several fungi and Oomycetes (Mares *et al.*, 2004). Companions were sown between the carrot rows either immediately after the carrots on 12 May 2008 or six weeks later on 18 June 2008 to attempt to reduce competition with carrot seedlings. The trial was designed as a split-plot design with four blocks. Each block consisted of five randomised subunits, one for each companion species. Each subunit comprised three beds: one control without companion plants (control), one bed with companion plants sown at the same time

as the carrots (early), and one with companion plants sown six weeks after the carrots (late). The three beds were randomised within their subunit. The layouts of both this and the 2009 trial are shown diagrammatically in the thesis.

In 2009 only wheat, barley and *Phacelia tanacetifolia* were included into the trial as companion species. In order to reduce competition between companion plants and carrots, the companion plants were sown before the carrots and removed early in the season.

The trial was designed as a lattice with four blocks, each comprising nine plots. Three controls were included per block to account for spatial variability in cavity spot disease observed in 2007 and 2008. The combinations of companion species and removal time were randomised with the three controls within each block. The companion plants were sown about six weeks before the carrots were drilled. They were either removed before the carrots were drilled (early) or six weeks after the carrots were sown (late).

Details of varieties, spacing *etc.* and sampling are given in the thesis.

2007 trial

In 2007 the first cavity spot disease in the carrots was detected at the first harvest on 26 July 2007 in the outer rows, but only one diseased carrot with three lesions. In the second harvest (6th until 14th August) no significant differences between any companion planting treatments were found. In the last three harvests the companion species planted between the carrots had a significant influence on the proportion of diseased carrots per sample (Table 6). In August and September the disease levels were below 10% in most treatments, except in clover and clover-grass mix. From September onwards the highest levels of cavity spot were found in clover and clover-grass mixture treatments, with higher disease levels than in the controls. Carrots grown with phacelia and barley had the lowest percentage of cavity spot disease in October and November, significantly lower than the levels found in the controls.

The proportions of diseased carrots were initially low in plots with carrots grown with ryegrass and leek, but increased between October and November and were not significantly lower than in the controls at the last harvest (Table 6).

The maximum numbers of lesions per diseased carrot increased over time for most treatments as the number of diseased carrots increased but throughout all harvests the majority of all carrots with cavity spot had three lesions or less. In carrots grown with ryegrass and *Phacelia tanacetifolia* (data not shown here) the lesion numbers per diseased carrot did not exceed four for any harvest. In plots with clover and clover-grass mix the proportion of carrots with more than three lesions per root increased steadily until

November/December, leading to a much flatter distribution of lesion numbers in the last two harvests than in the other treatments although the observed number of carrots with cavity spot was much greater than in the other treatments. In November/December most diseased carrots grown with clover-grass-mix had more than two lesions, whereas in all other treatment the majority of diseased carrots had only one or two lesions per root. In all other treatment and the controls only few carrots with more than four lesions were found in the last two harvests. The geometric means of the number of lesions per carrot did not vary significantly between companion treatments and the controls within the inner rows (data not shown).

Table 6: Proportion of carrots with cavity spot, inner rows. Harvests from August to November, companion trial 2007. Means were adjusted in REML, general linear model (Genstat, 10th edition). Homogenous groups apply to individual harvest times. P-values apply to factor "companion species" at α = 0.05.

Harvest	6 August	10 September	17 October	27 November
Companion	proportion diseased (%)	proportion diseased (%)	proportion diseased (%)	proportion diseased (%)
barley	6	4 ab	13 a	3 a
beetroot	3	9 ab	21 ab	40 cd
broccoli	5	8 ab	32 b	46 cd
clover	1	22 c	37 bc	53 d
clover-grass	5	12 b	52 c	83 e
Indian mustard	9	9 ab	23 ab	17 b
leek	6	3 ab	10 a	26 bc
nicotiana	3	3 ab	21 ab	19 b
phacelia	3	2 ab	11 a	5 ab
ryegrass	1	2 a	14 ab	23 bc
wheat	10	8 ab	18 ab	18 b
control	6	9 ab	31 b	36 c
SED	3.103	4.602	8.376	6.902
df res	32	32	32	32
Student's t	2.037	2.037	2.037	2.037
LSD	6.321	9.37	17.06	14.06
F pr.	0.11	0.01	< 0.01	< 0.01

Most companion plants competed strongly with the carrots, leading to yield loss of more than 50% in some combinations. In plots with barley, wheat, phacelia and ryegrass the carrot root weight was reduced strongly from the beginning of the season. Carrots grown with clover-grass mixture and clover appeared to be affected more strongly towards the end of the season than in the early season. Carrots grown with leek and *Nicotiana clevelandii* were not significantly lighter than carrots grown without companions (Table 7).

The effect of the distance between companion plants and carrots on the proportion of carrots with cavity spot was only significant in August and November/December, its interaction with

the companion species only in October (data not shown here). In August the proportion of diseased carrots was generally higher in the outer rows than in the inner rows, regardless of the companion species. In the following harvests the difference between outer and inner rows varied depending on the companion species. Carrots grown with clover and clover-grass mix and the controls had higher disease levels in the inner rows from September onwards, in barley and wheat more diseased carrots were found in the outer rows.

The distance between carrots and companions and the companion species had a strong effect on the carrot weight at all harvests, whereas the interaction between the two factors was not significant, except in September (data not shown). Carrots from the inner rows were generally lighter than carrots from the outer two rows. The competition from the companion plants was weaker in the outer carrot rows. In the outer rows the root weight of carrots grown with the most competitive companions did not differ as much from the weight of the controls as in the inner rows.

Correlations between root weight and the number of lesions per root and between root weight and the presence of cavity spot symptoms were very small, generally below 0.2. The correlations were similar for carrots from inner and outer rows and controls and all treatments (data not shown).

Table 7: Average root weight of carrots (g). Companion planting trial 2007, inner rows, July to November. Means were adjusted in REML, general linear model (Genstat, 10th edition). F pr. values apply to factor "companion species" at α = 0.05.

Harvest	18/7/07	6/8/07	10/9/07	17/10/07	27/11/07
Companion	weight (g)	weight (g)	weight (g)	weight (g)	weight (g)
barley	3 a	12 ab	27 ab	34 ab	32 ab
beetroot	11 bc	28 c	51 cd	55 bc	54 bc
broccoli	13 c	31 c	47 bc	65 cd	60 c
clover	13 c	27 c	36 b	43 b	46 b
clover-grass	8 b	16 b	23 a	27 a	29 a
Ind. mustard	11 bc	23 bc	50 c	57 c	54 bc
leek	15 cd	32 c	61 cd	69 cd	69 cd
nicotiana	16 cd	32 c	62 cd	77 d	71 cd
phacelia	5 ab	8 a	23 a	30 a	28 a
ryegrass	8 b	19 bc	25 ab	29 a	39 ab
wheat	4 a	11 ab	30 ab	38 ab	43 b
control	17 d	34 c	73 d	69 cd	78 d
SED	1.7	3.6	6.3	6.1	6.7
df. res.	32	32	32	32	32
Student's t	2.037	2.037	2.037	2.037	2.037
LSD	3.4	7.3	12. 8	12.3	13.7
F pr.	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

In 2007 samples of carrot surface soil were tested for *P. violae*-DNA by end-point PCR. In July and August *P. violae* was not detectable in surface soil of carrots grown with phacelia, clover and leek. From September onwards it was found in samples from all treatments. In November *P. violae* was found in at least three of four repeats for all treatments, except phacelia and wheat, where *P. violae* was found in only one of four repeats. No companion planting treatment resulted in the elimination of *P. violae* from root surface soil. In 2007 the samples of root surface soil from the last two harvests in October and November, but not from the previous harvests, were also tested by qPCR. In October the amounts of DNA of *P. violae* were either undeterminable or around the equivalent of one diploid genome (0.04 - 0.09 pg) per mg soil in most samples of carrot root surface soil. Higher levels were only found in root surface soil of carrots grown with clover, clover-grass and nicotiana. In November the levels were generally higher with more than one genome of *P. violae* found in at least one sample from each treatment, except wheat (Figure 8).

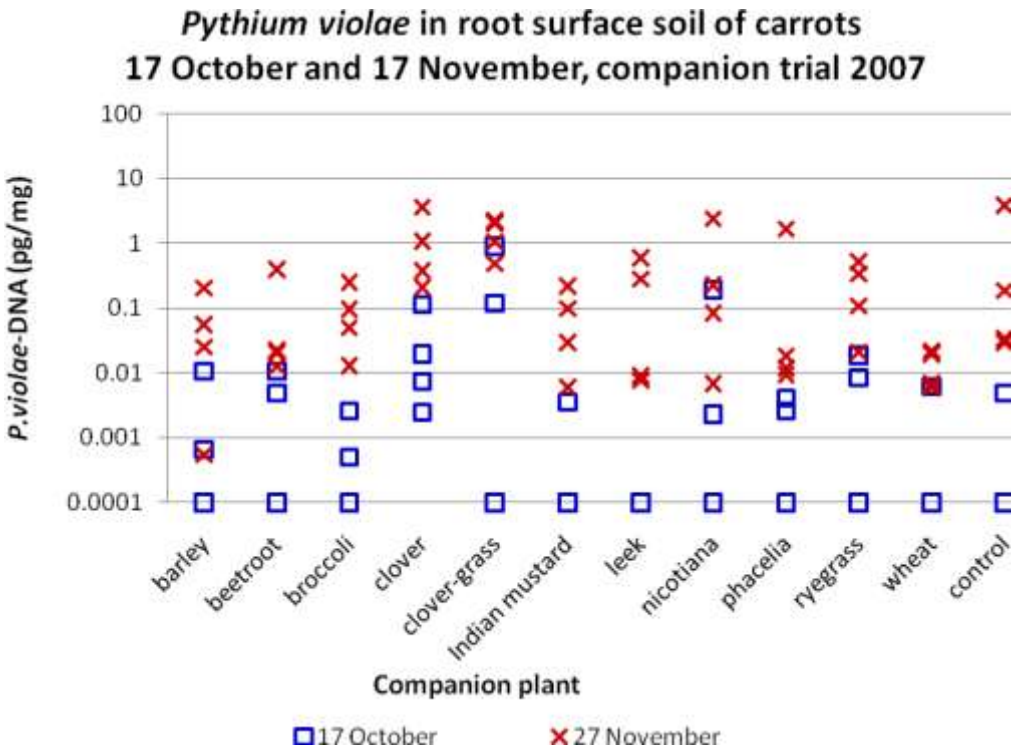

Figure 8: DNA of *Pythium violae* in root surface soil of carrots from inner rows. Companion plant trial, 17 October and 27 November 2007.

2008 trial

Although companion planting reduced cavity spot in 2007 with most species used, reduced carrot yield due to competition was a major problem. In 2008 an attempt was made to reduce the effects of competition by planting the companions some weeks after the carrots were drilled. Only 5 “companion” species were used.

In the first harvest the number of diseased carrots was not significantly affected by sowing time of companions or by the companion species. In the second harvest only the sowing time had a significant effect. In plots where companions were sown early the number of diseased carrots was lower, except in plots planted with clover as a companion (Table 8). In the third harvest the companion species in interaction with sowing time affected the number of diseased carrots significantly. In all treatments with early sown companion plants fewer diseased carrots were found than in the controls. The effect was strongest in plots with phacelia and marigold as companion plants. The only exception were plots undersown with clover, in these plots the number of carrots with cavity spot was not significantly different

from the controls, regardless of sowing time. Companions sown 6 weeks after the carrots did not affect the proportion of affected carrots.

The geometric means of the number of lesions per root varied by one lesion or less between treatments. Only in plots with early sown phacelia was a consistent reduction in lesion number per root over all harvests observed (data not shown). The variation between the control plots was similar to the variation between treatment combinations.

Competition between carrots and companions was strongest when companions were sown at the same time as carrots. Carrots in these plots were significantly lighter than carrots from control plots and plots where companion plants were sown after carrots. Carrots grown with early sown phacelia had the smallest root weight in all harvests, followed by the carrots grown with early sown barley or wheat. When the companions were sown six weeks after the carrots, no significant carrot root weight reduction was observed. The only exception was clover. The root weight of carrots grown with clover was not significantly different from the controls even for clover sown at the time the carrots were drilled

Correlations between root weight and the number of lesions and root weight and cavity spot disease were statistically significant but very small, mostly below 0.2, in all harvests (data not shown).

The levels of *P. violae* DNA in open soil were low before sowing in April. Only up to about the equivalent of one genome of *P. violae* per mg soil was detected in most samples. The amount of *P. violae*-DNA in carrot surface soil was higher in all harvests than the levels in open soil before sowing in all companion treatments and controls. The majority of samples from carrot surface soil contained more than one genome of *P. violae* and up to ten times as much *P. violae*-DNA than the open soil samples. The variation between the samples within each treatment was usually higher than the differences between treatments. The detectable DNA levels did not increase between harvests (data not shown). There was no clear and

consistent effect of companion plant on levels of *P. violae*, whether the companion was planted at the time of drilling or later.

Table 8: Upper - proportion (%) of diseased carrots in companion trial 2008, July to September. Lower - tests of statistical significance for each harvest. LSDs are supplied when combination effect was significant. SED and LSD values in brackets apply to comparison within same level at α = 0.05. Homogenous groups: Upper case: times of sowing compared to control for each companion species. Lower case: companion plants or controls within one sowing time.

Harvest	Sowing time / Companion	early	late	no companion
		Proportion (%) diseased carrots	Proportion (%) diseased carrots	Proportion (%) diseased carrots
22/7/08	barley	20	13	22
	clover	25	13	16
	phacelia	8	9	18
	marigold	5	17	15
	wheat	12	16	13
19/8/08	barley	21	33	37
	clover	35	32	32
	phacelia	3	34	33
	marigold	10	19	35
	wheat	14	37	41
22/9/08	barley	18 a A	42 b B	41 a B
	clover	46 b A	29 ab A	29 a A
	phacelia	5 a A	36 ab B	29 a B
	marigold	8 a A	21 a B	30 a B
	wheat	15 a A	51 b B	38 a B

Harvest	Factor Measure	companion	sowing time	combined
22/7/08	F pr.	0.6	0.2	0.5
	SED	4.5	2.7	6.7 (6.1)
	df. res.			30
19/8/08	F pr.	0.1	< 0.01	0.2
	SED	4.7	4.3	9.2 (9.7)
	df. res.			30
22/9/08	F pr.	< 0.01	< 0.01	< 0.01
	SED	3.9	3.9	8.2 (8.8)
	LSD	8.4	8.0	16.5 (17.9)
	df. res.			30

2009 companion trial

In 2008, planting companion species at the same time as the carrots had given good control of cavity spot, but unacceptable levels of competition, whilst planting them 6 weeks after the carrots had given no control. Therefore in 2009 the companions were planted before the carrots and removed either at the time of drilling the carrots or six weeks later.

The proportion of diseased carrots did not differ significantly between any treatment combinations and the controls in early August when less than 10 % of carrots had any cavity spot lesions. In the following two harvests only carrots which had been grown with companions for the first six weeks showed significantly reduced disease levels, independent from the companion species. Disease levels in carrots whose companion plants were removed at carrot drilling were not different from the controls (Table 9).

Table 9: Proportion of diseased carrots, companion trial, August to October 2009. Companion plants were removed at carrot drilling (early) or six weeks after drilling (late). No companion plants were planted in control plots. Each treatment combination was replicated 4 times, the controls 12 times. Means calculated in ANOVA. SEDs provided for comparison of treatment combination means at α = 0.05.

Harvest	Removal time	early	late				
	Companion plant	% diseased carrots	% diseased carrots	Measure	companion	removal time	combined
3/8/09	barley	5	0	F pr.	0.43	0.10	0.43
	phacelia	0	0				
	wheat	3	0	SED [1]	2.8		
				SED [2]	2.3		
	control	5		df. res.			26
24/8/09	barley	19	0	F pr.	0.80	< 0.01	0.88
	phacelia	17	0				
	wheat	25	1	SED [1]	10.4		
				SED [2]	8.5		
	control	18		df. res.			26
5/10/09	barley	31	2	F pr.	0.77	< 0.01	0.48
	phacelia	41	2				
	wheat	30	8	SED [1]	10.2		
				SED [2]	8.3		
	control	32		df. res.			26

[1] applies to comparisons between treatment combinations
[2] applies to comparisons between controls and treatment combinations

The frequency distributions of lesion numbers in the controls and the plots from which the companion plants barley or wheat had been removed early were similar in all harvests (data not shown). In plots where phacelia had been grown until after carrot seedling emergence no

diseased carrots were found at the first harvest, but in the following two harvests the distributions of lesions numbers were similar to those observed in the controls. In treatments in which the companion plants had been removed six weeks after carrot drilling, no diseased carrots were found at the first harvest. At the second harvest only one diseased carrot with two lesions was found in a plot with wheat as a companion plant and none in the other treatments. In the third harvest the number of diseased carrots was still lower than in the plots where the companions had been removed at carrot drilling. The number of diseased carrots was too low to compare the distributions of lesion numbers with the other treatments and the controls.

In all observed distributions the majority of the diseased carrots had up to four lesions. The proportion of carrots with more than four lesions increased over time as the number of diseased carrots increased. The geometric means of the lesion numbers per diseased carrot were not analysed in an ANOVA because of the low number of observations in some of the treatments.

The carrot root weight was reduced significantly in plots where the “companion” plants had been left to grow alongside the carrots for the first six weeks (data not shown). By October the roots which had grown alongside “companions” for the first six weeks were still approximately 50 % lighter than the controls, even though the carrots had been without companions for two months. No root weight reduction in comparison to the controls was observed when the companions were removed at carrot drilling.

The correlations between root weight and cavity spot disease and root weight and the number of lesions were very small in all harvests. The correlations were generally smaller for the controls than for all harvested carrots.

Pythium violae was only detected in two samples of open soil before sowing. On 3rd August, *P. violae* was found in root surface soil of carrots from the controls and from plots where the companion plants had been removed at drilling. *P. violae* was found in only one sample from plots where the “companion” plants had been removed six weeks after drilling. DNA amounts varied more within each treatment than they differed between samples from different treatments. On 24 August *P. violae* was found in most samples from plots where “companion” plants had been removed early. The detected amounts in plots with early removed barley and phacelia were similar to those found in the controls and higher than the amounts detected in plots with late removed “companion” plants. For early removed wheat the range of detected DNA amounts was similar to the amounts detected in plots where the “companion” plants had been removed six weeks after drilling.

On 5th October the range of amounts of *P. violae*-DNA in carrot surface soil was greater than in the first two harvests (Figure 9). The range of amounts found on carrots which had been grown with "companion" plants for the first six weeks was slightly lower than the amounts detected on carrots from the control plots and plots where "companions" had been removed early. The exception was wheat, for which the ranges were similar for early and late removal. The range of amounts of *P. violae*-DNA found on carrots from plots with early removed wheat was lower than in plots with early removed phacelia and barley. The range of amounts of *P. violae*-DNA detected on carrots from the control plots was wider than the differences between treatments and the control.

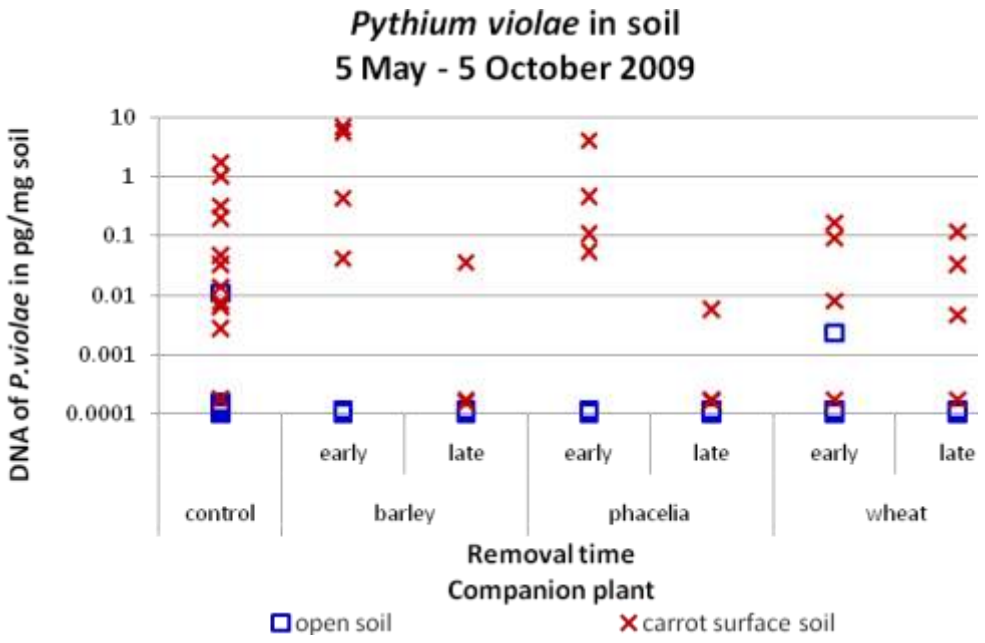

Figure 9: DNA of *Pythium violae* in open soil before sowing and carrot root surface soil at third harvest (5 October), companion trial 2009. Companions were removed at same time as carrots were sown (early) or six weeks after drilling the carrots (late). No companion plants were sown in control plots. Replication: 12 for control, 4 per treatment combination.

IV: Spatial distribution of *P. violae*

Aims of the experiments

In general soil-borne diseases are unevenly distributed in the soil, and this is true of cavity spot. However the precise distribution has been little studied. In designing experiments to study soil-borne diseases it is important to understand the scale of the variation to properly allow for its effects. Moreover, understanding the distribution of the disease will allow insights into the mechanisms of disease spread. The nature of this data meant that precise statistical analysis was difficult and the primary approach was visual examination of diagrammatically displayed data.

Companion plant trials

In all three trials the distribution of cavity spot across the sampled fields was patchy, particularly in the early samplings. The incidence of cavity spot varied with the companion planting treatment as well as within each level of treatment. The proportion of carrots with cavity spot varied greatly amongst repeats of some treatments in the companion trials. For example, in the last harvest of the first companion planting trial in November 2007 the proportion of diseased carrots in plots with Indian mustard as a companion the cavity spot incidence ranged from below 10% to between 31% and 40%. The variation within the controls was greater, the cavity spot incidence in the controls ranged from below 10 % in block 3 to above 75% in block 4. The incidence of cavity spot did not always increase within one plot between samplings, suggesting that the incidence of cavity spot also varied within plots. In August between 21% and 30% of all sampled carrots from the wheat-carrot-plot in block 3 were diseased whereas in September less than 10% diseased carrots were found in the carrots sampled from this plot. Within the trials no strong gradients in the spatial distribution of cavity spot incidence were observed, indicating that generally the treatment effects were greater than the effects of large-scale variations in the spatial distribution of cavity spot disease. However, in the 2009 trial the incidence of cavity spot appeared to be somewhat increased in a band across the field, running vertically from the boundary between block 1 (top left) and block 2 (top right) towards block 3 (bottom left); this is most easily appreciated by comparing control plots (i.e. carrots with no companion). This pattern appeared to be at least partly independent from the companion planting treatments (most data not shown but for the 2009 trial see Figure 10).

Overall it seemed that the scale at which these experiments had been designed were sufficient to show major effects of the companion planting but that the effects of non-random distribution of the disease prevented a fine level of interpretation of the data.

Rotation trials.

As with the companion plant the distribution of inoculum and final distribution of the disease varied across the trial. This is easiest seen in the first 2 year rotation (Figures 11 & 12). However, again it appeared that the plot size and level of replication was sufficient to allow major effects to be seen but not sufficient to assess small potential differences between treatments.

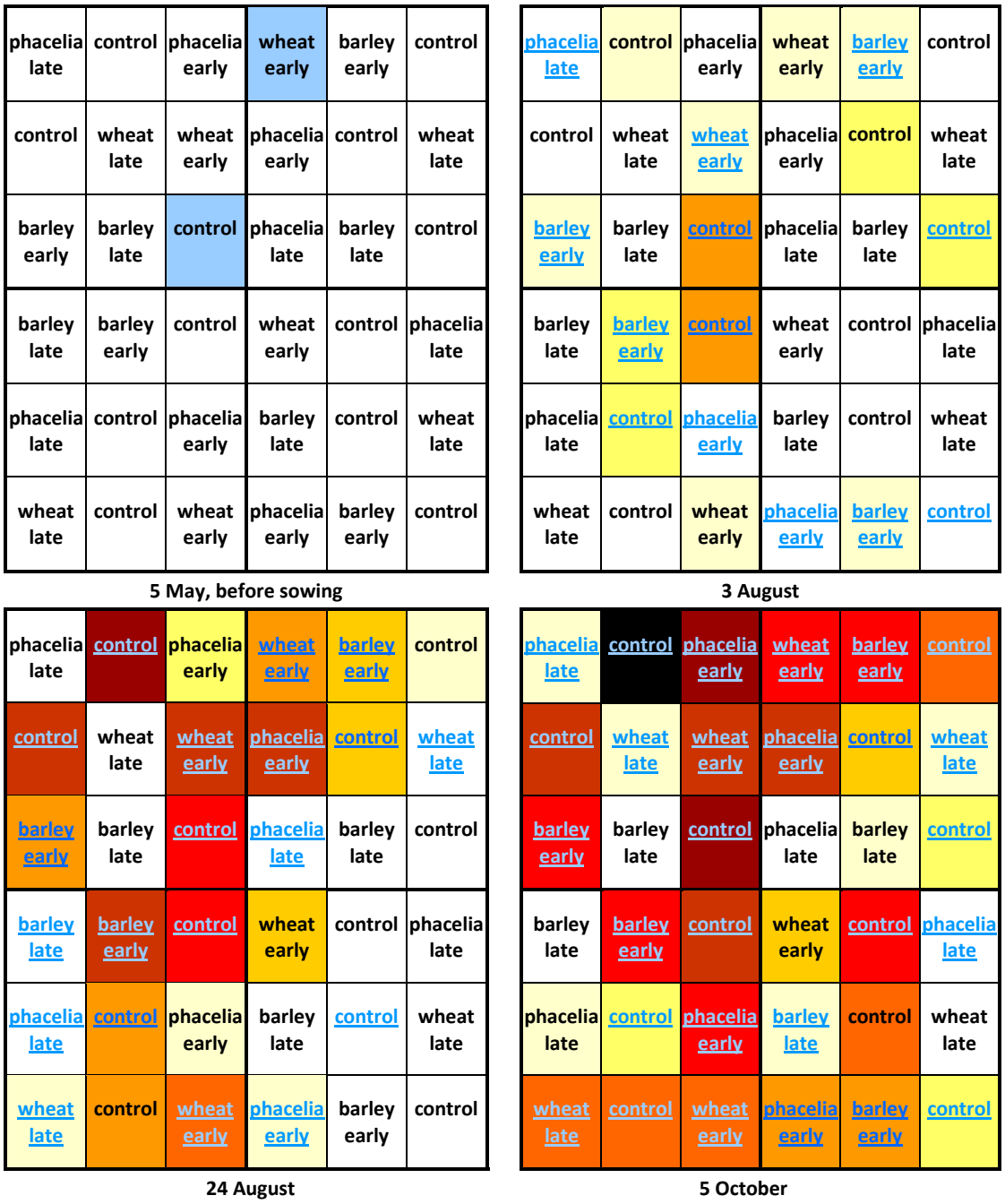

Figure 10: Companion trial 2009, distribution of *P. violae* in soil (blue), detected by qPCR before sowing in May (top, left) and cavity spot incidence (% diseased carrots) from early August (top right) to early October (bottom right). Companion species and companion removal time are named in each plot. Carrots without companion plants were grown in control plots. *P. violae* was detected in carrot surface soil at harvest by qPCR in plots with treatment names in blue and underlined. Bold lines mark block boundaries. Plot sizes in graph are not to scale with field plots. Intensity of colour indicates cavity spot incidence from white (0%) to the darkest (76-100%)

barley 2.1	carrot 2.1	clover 2.1	broccoli 3	potato 3	clover grass 3	rape 2.2	sugar beet 2.2	wheat 2.2
broccoli 2.1	clover grass 2.1	rape 2.1	sugar beet 3	carrot 3	wheat 3	barley 2.2	clover 2.2	potato 2.2
wheat 2.1	sugar beet 2.1	potato 2.1	barley 3	rape 3	clover 3	clover grass 2.2	broccoli 2.2	carrot 2.2
clover grass 2.2	barley 2.2	wheat 2.2	carrot 2.1	sugar beet 2.1	potato 2.1	broccoli 3	rape 3	clover 2
clover 2.2	potato 2.2	sugar beet 2.2	rape 2.1	clover grass 2.1	broccoli 2.1	carrot 3	wheat 3	barley 3
carrot 2.2	rape 2.2	broccoli	wheat 2.1	clover 2.1	barley 2.1	potato 3	clover grass 3	sugar beet 3
rape 3	clover 3	carrot 3	clover grass 2.2	barley 2.2	sugar beet 2.2	wheat 2.1	potato 2.1	broccoli 2.1
sugar beet 3	broccoli 3	barley 3	potato 2.2	wheat 2.2	rape 2.2	clover 2.1	carrot 2.1	clover grass 2.1
potato 3	wheat 3	clover grass 3	clover 2.2	broccoli 2.2	carrot 2.2	sugar beet 2.1	barley 2.1	rape 2.1

Figure 11: *P. violae* in open soil before sowing the rotation trial, 18 April 2008. Plots with bold treatment names were sown with carrots in 2008. The plots with light grey labels were sown with carrots in 2009. In plots shaded with dark blue or light blue *P. violae* was detected in soil by qPCR before sowing the carrots (dark blue) or before the last set of alternative crops preceding carrot due to be sown in 2009 (light blue). Plot sizes in graph are not to scale with field plots. Compare the distribution of inoculum in the 3 blocks sown with carrots in 2008 with the distribution of disease (Figure 12, below).

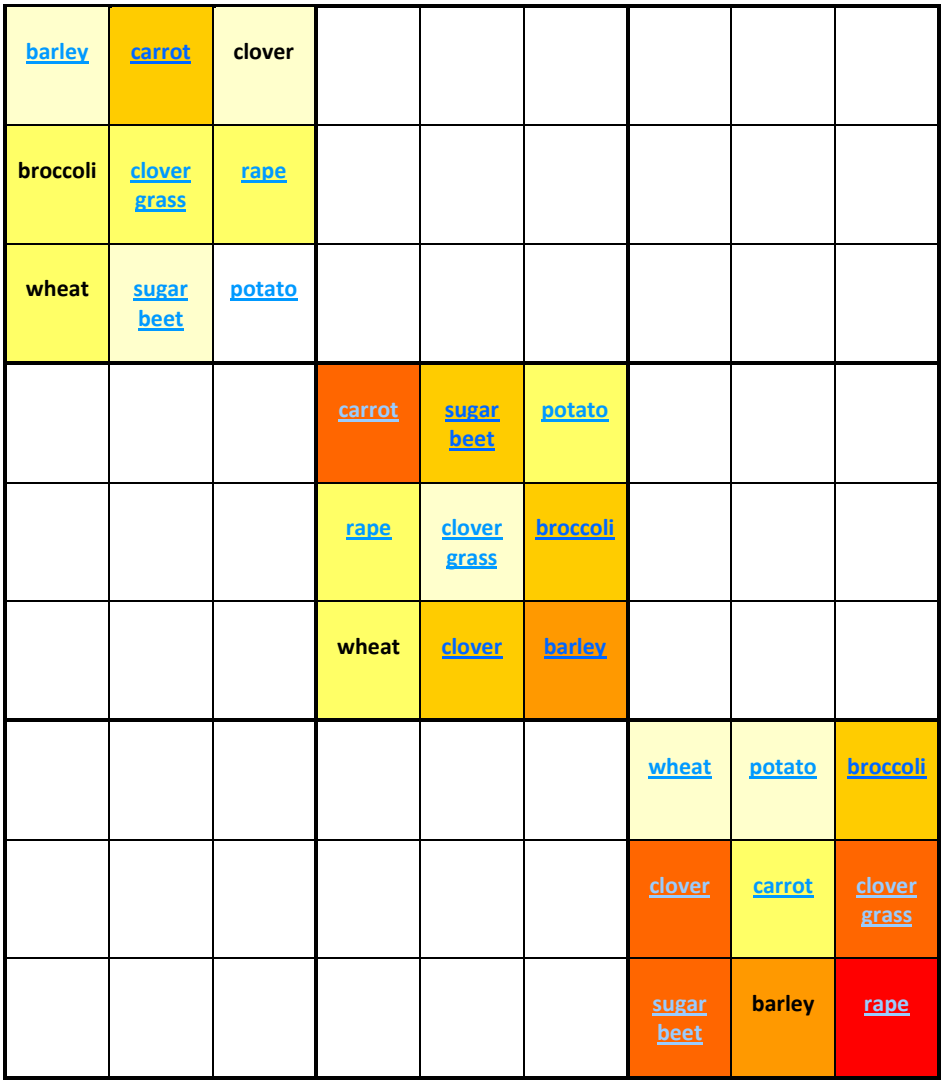

Figure 12: Distribution of cavity spot incidence (% diseased carrots) across field in the first two-year rotation trial, 7 October 2008. The crop preceding the sampled carrots are named in the plots. In plots with treatment names blue and underlined *P. violae* was detected in carrot surface soil at harvest by qPCR. Bold lines mark block boundaries. Plot sizes in graph are not to scale with field plots. Fill colour reflects incidence of disease from white (0%) to the darkest shade used here (21-30%)

Within row (fine scale) distribution of cavity spot.

The fact that within trials, incidence of disease did not always change smoothly between sampling times suggested small scale heterogeneity in the distribution of infection and disease. To study this, the position of every carrot within three 2.5m rows was determined, the carrots lifted, washed and the numbers of lesion counted. Two rows were from the 1st 2-year rotation trial and the third from the 3-year rotation trial. A smaller section (1 m) of one of these rows is shown in Figure 13.

Diseased carrots were not uniformly distributed within the sampled carrot rows (*e.g.* Figure 13). Carrots with more than four lesions were mostly found within clusters of other diseased carrots, whereas carrots with four or fewer lesions appeared singly amongst healthy carrots as well as in clusters of diseased carrots. This clustering of carrots with high numbers of lesions was most apparent in the row with the densest stand of carrot roots from the edge of a carrot-broccoli plot (data not shown here). In the more sparsely populated rows, carrots with more than four lesions were found isolated amongst healthy carrots as well as within clusters of diseased carrots (data not shown here). This clustering deviated significantly from a random distribution (Table 10).

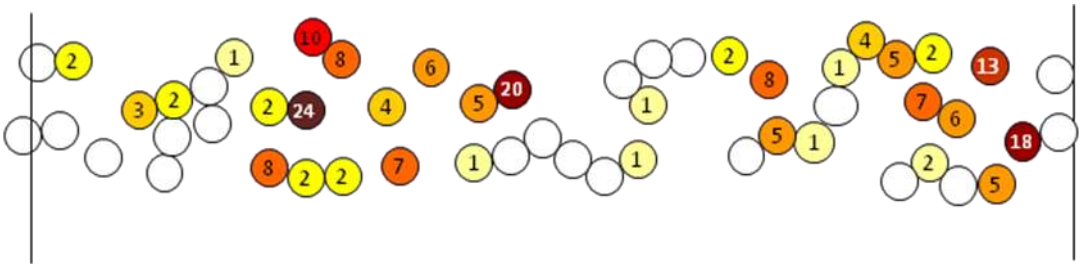

Figure 13: Number of lesions per carrot in a row (1 metre of the left edge row from the first companion planting trial, block 2, companion plant beetroot) sampled on 18 February 2008. This is part of the complete 2.5 meter row actually sampled shown for illustrative purposes. Intensity of colour reflects number of lesions on individual roots with the number in circle being the exact number of lesions found on individuals.

Table 10: Spearman's rank correlation coefficients (r_s) for the number of lesions per carrot and parameters of disease severity in neighbouring carrot roots. Three rows of carrots from plot edges were analysed.

Row	carrot-broccoli 2008		carrot-beetroot 2008		carrot control 2009	
Value	r_s	P-value	r_s	P- value	r_s	P-value
diseased roots among closest 5 roots	0.43	0.00	0.40	0.00	0.44	0.00
diseased roots among closest 10 roots	0.51	0.00	0.42	0.00	0.37	0.00
total lesions on 5 closest roots	0.49	0.00	0.46	0.00	0.38	0.00
total lesions on 10 closest roots	0.53	0.00	0.43	0.00	0.31	0.00
highest lesion number in cluster with 5 closest roots[1]	0.61	0.00	0.55	0.00	0.46	0.00
highest lesion number in cluster with 10 closest roots[1]	0.56	0.00	0.43	0.00	0.36	0.00
Sample size:	172		144		115	
Degree of freedom (df.):	170		142		113	

[1] The cluster includes the tested root itself, *e.g.* if highest number of lesions per root amongst the closest five adjacent roots is 4 and the sampled root itself has 7 lesions, the value is 7.

A cluster of 24 carrots composed of both carrots with cavity spot lesions and healthy appearing carrots without cavity spot lesions was tested for the presence of *P. violae*. DNA of *Pythium violae* was found in the root surface soil of all but two diseased carrots in the cluster and also on four of ten healthy carrots (Figure 14). All healthy carrots in whose surface soil *Pythium violae* was found grew adjacent to at least one carrot with cavity spot lesions. Most healthy carrots which were tested negative were growing isolated or among roots without cavity spot symptoms. Spearman's rank correlation between the number of lesions per root and the amount of *P. violae*-DNA in root surface soil was weak (r_s 0.46), but statistically significant (P-value 0.02) at $\alpha = 0.05$. The amounts of *P. violae*-DNA varied strongly between positive tested samples.

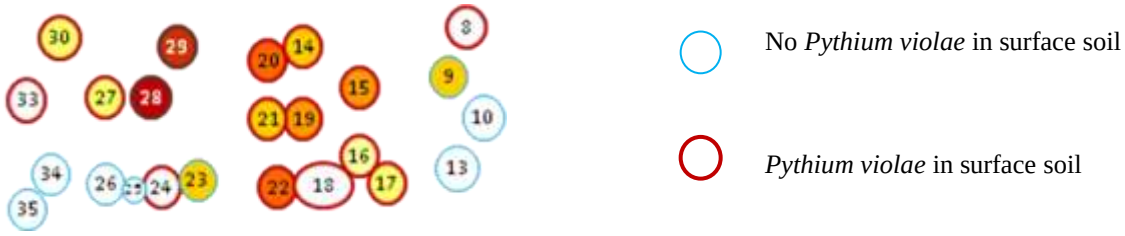

Figure 14: Cluster of sampled carrots from control, companion planting trial, 2009. Shading of circles indicates number of lesions. Bold red circles indicate that *P. violae* was detected in root surface soil by qPCR, blue circles indicate a negative result.

V: Growth and oospore production of *P. violae* and resistance tests of carrots against cavity spot

A series of experiments were aimed at understanding the growth and spread of *Pythium violae* in soil and *in vitro* and the production and germination of oospores. Further experiments aimed at developing or improving methods of soil and root inoculation are also described as well as approaches for future research projects.

Although described in detail in the thesis, most of these experiments should be considered preliminary because they were done on a small scale. The aim of these experiments was to provide a basis for more extensive research which could not be completed within the three years of this PhD-project. These experiments will not be reported here as they currently have limited practical applicability.

Discussion

Distribution of disease.

Cavity spot disease was clearly not evenly distributed within individual rows nor between plots of the same treatment in the various field trials. However, there was only very limited evidence of heterogeneity at the scale of whole trials. Heterogeneity in distribution is a common facet of soil-borne diseases (as the inoculum does not spread easily or quickly) and trials here were designed at a scale and level of replication intended to cope with the expected heterogeneity. The broad conclusion was that the trials designs were sufficiently robust to allow study of large treatment effects but that the heterogeneity introduced too much variation between individual samples from within plots and between plots to allow study of small effects. As at the outset it had been hoped to find treatments in the rotation and companion plant trials giving at least 50% changes in levels of disease these designs proved in retrospect adequate for purpose.

Rotation trials.

As noted above the heterogeneity in disease distribution prevented fine scale differentiation of treatments. Potatoes seemed the most effective crop in reducing disease with reductions of up to 75% (although in the second two-year rotation, where overhead rather than trickle irrigation, was used when the potatoes were being grown the reduction was only 20% - possibly emphasising the need to have well-grown crops in the rotations). Several other crops (broccoli, clover, clover-grass mix) consistently gave lower levels of disease than the controls but these reductions were only significant in the three year (crop-crop-carrot) rotation. Three crops (barley, sugar beet, wheat) did not consistently reduce or increase disease. One crop (forage rape) increased disease in all three (two two-year and one three-year) trials; this was possibly a real-effect but the high level of variability between plots meant this was significant in only one of the three trials.

Carrot after carrot (the controls) gave generally low root weights but the other rotations had similar root weights except for possibly broccoli in the second two-year rotation (although not in the other two trials). Within these limited trials it cannot be said that any of the rotations had an effect on total yield, relative to what would have been expected for first year carrots.

The effects on disease were not paralleled by effects on the amounts of *P. violae* detectable in soil from carrot surfaces nor in effects on soil inoculum. We have found in other projects that there is little relationship between numbers of lesions and amount of *P. violae* (except at high numbers of lesions). As in these other projects, the effect of rotations seems to be on

the “efficiency” with which the fungus causes disease rather than by a simple reduction in amount of fungus. This will be discussed further later.

“Companion planting”.

The different “companions” tried in the first trial had range of effects, five significantly reduced disease at the November harvest, two increased it (clover and clover/grass but ryegrass alone had no significant effect and we presume that the clover was the “active ingredient in the mixture) and four had no statistically significant effect. Therefore, this trial clearly demonstrated that the presence of other plants among the carrots can affect development of the disease. But, and it is a big but, disease reduction came at the cost of a major reduction in yield. The other two trials therefore had two aims *viz.* (i) to confirm that disease levels can be affected by “companions” and look at the consistency of this and (ii) to adjust the relative planting removal dates to attempt to minimise competition.

In 2008, drilling the “companions” at the same time as the carrots confirmed that there was an effect on disease; four significantly decreased disease and clover increased disease by 60% (but this latter was not statistically significant). However, when the companions were sown six weeks after the carrots no effect on disease levels were seen, although this did reduce competition. This was a first indication that a period up to six weeks at the start of the carrot crop appears to be crucial to reducing disease.

In 2009, the three “companions” used were sown six weeks before the carrots and removed either at the time the carrots were drilled (“early”) or six weeks later (“late”). Removing the “companions” early meant that there was no competition with the crop but also no effect on disease. Removing them late gave very good levels of disease control (at worst a 75% reduction in October, the last harvest, for wheat) and over 90% for barley and phacelia. However, by the October harvest there was still a 50% reduction in yield for the late removed “companions” even though the competing plants had been gone for over two months.This trial again emphasised that some early period (of up to 6 weeks) is crucial to disease development.

The effects on levels of fungus in the soil were less clear. It did appear that there was some reduction in the amount of fungus detectable on the carrot roots at the end of the season where disease was also reduced (particularly in the 2009 trial) and where the presence of clover lead to an increase in disease this gave a slight increase in detectable late season fungus.

From these trials it is clear that “companion” planting can lead to a reduction in cavity spot but that competition with the crop can be a major problem. It is also clear that some period within the first six weeks is crucial to the disease suppression.

Whilst varying the timings resulted in reduction in competition, further work needs to be done on this to achieve a commercially acceptable system. One of the most successful "companions" was barley. This crop is already used as a "nurse" crop for carrots by some growers. For this purpose, the barley is drilled at or around the same time as the carrots and removed within a few weeks. As the crucial period for disease control seems to be during the first six weeks but it is not clear whether the "companion" needs to be partly grown at the time the carrots are drilled and how long after drilling they need to be present. The effectiveness of barley in suppressing disease means that further work is warranted.

Hosts and mechanisms.

P. violae has been known to have a wider host range than *P. sulcatum* (van der Plaats-Niterink, 1981) and it was found here that at least some plants of most wild and cultivated species will support the growth of *P. violae.* Some species seemed better hosts than others but this should be interpreted with care. In the case of natural infections it is difficult to separate varying suitability as a host from the non-homogenous inoculum in the soil and for the pot experiments the absence of a competing micro-biota may affect the out-come. What is clear, however, is that *P. violae* is not exclusively dependent on carrots for its maintenance. The corollary of this is that not having grown carrots in a particular field, either ever or for a long-time, is not likely to mean that the field will be free of *P. violae* and cavity spot disease.

What the mechanism or mechanisms of the effects in the rotation and "companion" plant trial is/are not clear. Where direct comparisons of single species in the two types of trial can be made, the effects seem contradictory. Barley was one of the best two species of "companion" plants but had little effect when grown as a rotation for one or two seasons prior to carrots. Clover and clover/grass mixture increased disease as a "companion" but decreased it as a rotation crop (although less then potato). Neither barley nor clover were the best hosts of *P. violae* (in the sense of being most often infected or supporting higher levels of the fungus than other species) but neither were they non-hosts. Potato, which was the most effective species for reducing disease in the rotation trial, was a poor host of the fungus, but again not a non-host. There was also no evidence that it reduced the amount of fungus on the surfaces of carrots, and if anything it gave the highest levels of *P. violae* left in the soil after the second pre-carrot crop (Fig. 4).

In the "companion" plant trials there was evidence that the levels of fungus on the carrot roots were affected. In the two trials where clover was used the level of *P. violae* was the highest. In the last (2009) trial barley and phacelia reduced the levels of the pathogen in treatments where disease was reduced (but not in those where disease was unaffected) but

this was presumably not a direct antibiosis as both species seemed to support the growth of the fungus in or on their own roots.

Taken with evidence from other projects, possibly the best explanation is that many of the effects in both the rotation and "companion" trials were due to complex effects on the total soil microbiota which had "knock-on" effects on the fungus and its capacity to cause disease. However, this is very tentative and further work will be needed to truly understand what is going on in these trials

Conclusions

- Inoculum is dispersed heterogeneously in the soil but the trials were carried out on a suitable scale to allow effects to be seen.
- *P. violae* has a broad host range amongst both wild (weed) and cultivated species which means that long rotations out of carrots will not ensure freedom from cavity spot.
- Pre-carrot rotations can affect levels of cavity spot disease
    - Potato gave reductions of 65-75% in two of the three trials (but potato is unpopular with growers due to the problem of controlling volunteers during the carrot crop)
    - Most crops were neutral or gave lower reductions in disease levels but one, forage rape, increased the disease (although only statistically significantly in one trial).
- "Companion" planting can affect the levels of cavity spot disease
    - A period within the first six weeks of the crop is crucial to this effect.
    - Unfortunately, to date the competition with the drop has given unacceptably high yield losses.
    - Barley was one of two most effective "companions" – this crop is already used as a nurse crop by some growers without unacceptable losses and further studies on relative planting times may produce a commercially acceptable system.

Technology transfer

Two papers in scientific journals are being prepared based on this thesis.

International Conferences.

- Anne Kretzschmar attended the 32nd International Carrot Conference in Arcachon, France. 5th - 6th September 2007 and discussed her work with other attendees.

- Anne Kretzschmar attended the 33rd International Carrot Conference in Anaheim, California in 18th - 21st January 2009. Poster presented: The role of non-carrot crops and wild plants in the epidemiology of cavity spot (*Pythium violae*).

Short reports in HDC News:

- October 2007 (p18) CP46 Carrot cavity spot: the effects of non-carrot crops on levels of relevant pythium species in the soil. (As part of "Youth Culture" where Spence Gunn outlines current HDC studentships)
- Can Companions control cavity spot? Page 18, No 158 (November 2009) – part of article on current HDC studentships.
- More revealed about the secret life of carrot cavity spot (New annual report available for CP46). Page 10. No 153 (May 2009)

Feature articles in HDC News:

- October 2007 (pp 20-21) - Opening cavity spot options. (Described progress in DEFRA HH3230SFV and two HDC projects, CP 46 & FV 5g)
- Article submitted for publication 2010: Cavity Spot of Carrots. (Describes results from CP 46 and FV 353)

Presentations at grower events:

- D.J. Barbara. HDC (BCGA) Carrot Technical Seminar. PGRO Thornhaugh, Cambs. Thursday 25th January 2007. Carrot Cavity Spot (CP 46 & FV 5g)
- D.J. Barbara. Syngenta Carrot Agronomy meeting. Thursday 29th March 2007. Norton Lodge Conference Centre, Old Harbour Farm, Norton Disney, Lincoln, LN6 9JR. Cavity Spot, the latest research findings, future directions and possible applications. (Described progress in DEFRA HH3230SFV and two HDC projects, CP 46 & FV 5g,)
- D.J. Barbara. Vegetable Consultants Association. 31st July 2007. Charlecote Pheasant, Wellesbourne. Cavity spot in carrots. (Described progress in DEFRA HH3230SFV and two HDC projects, CP 46 & FV 5g,)
- D.J. Barbara. Review of cavity spot at Warwick HRI. British Carrot Growers Association, R&D committee. Thursday 1st November 2007 (Described progress in DEFRA HH3230SFV and two HDC projects, CP 46 & FV 5g,)
- D.J. Barbara. Carrot Cavity Spot: where are we and where might we go? Onion and Carrot conference, East Of England Showground; Thursday 22nd November. 2007 (Described progress in DEFRA HH3230SFV and two HDC projects, CP 46 & FV 5g,)
- D. J. Barbara. British Carrot Growers Association, R&D committee.15th Jan 2009. General description of progress with cavity spot.

- D. J. Barbara. ADAS/Syngenta carrot symposium, 29th Jan, 2009 (Briefly mentioned CP 46)
- D. J. Barbara. BCGA Technical Seminar 19th March, 2009 Understanding *Pythium violae*, the cause of carrot cavity spot in the UK. (Briefly mentioned CP 46)
- D. J. Barbara. HDC/BCGA Carrot technical Seminar 22nd April 2010 at PGRO. (Described progress in DEFRA HH3230SFV and two HDC projects, CP 46 & FV 5g,)

Other:

- Anne Kretzschmar. Effect of non-carrot crops on cavity spot in carrots and soil inoculum of *Pythium violae*. Presentation at Warwick HRI postgraduate symposium 7/8 May 2009.
- Anne Kretzschmar. Influence of undersown crops on the severity of cavity spot on carrots. Poster at Warwick HRI postgraduate symposium 17/18 March 2008.

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