August 2013

# Project Report No. 512

## Importance and management of Verticillium wilt in winter oilseed rape

by

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This is the final report of a 36 month project (RD-2009-3618) which started in January 2010. The work was funded by Farmacy/Hutchinsons and a contract for £99,997 from HGCA.

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## 1. ABSTRACT

The aim of this project was to establish the importance of verticillium wilt in winter oilseed rape and carry out initial work on varietal resistance and the impact of agronomic factors.

Between 2009 and 2011, verticillium wilt was confirmed in 16% of 292 randomly selected crops of winter oilseed rape that were sampled in late June. About 5% of crops had more than 30% of plants affected and the average level of verticillium wilt in England was 3.3% of plants affected. Verticillium wilt was found most frequently in eastern England, but it occurred in all regions and as far north as Yorkshire.

There were significant differences in verticillium wilt severity between winter oilseed rape varieties with widely grown varieties such as Castille, Excalibur and Es Astrid being amongst the most susceptible. The varieties Catana, Oracle, Alienor and Compass were considered to have useful levels of resistance though further testing of varieties is required to substantiate these initial results.

The effect of verticillium wilt on yield was investigated by hand harvesting plants from four commercial crops with different severities of verticillium wilt. Yield loss was evident when verticillium wilt was severe and was due to decreased thousand seed weight, estimated at 12-24% in severely affected plants. Experiments on date of sowing and soil temperature, together with survey data, indicated that verticillium wilt can develop under a wide range of temperature conditions. Symptoms of verticillium wilt appear to be more severe when plants are not growing vigorously or are under stress. Thus any potential benefit from delaying sowing to reduce verticillium infection is offset by greater symptom expression on small plants.

Efforts to develop a DNA-based soil diagnostic test for *Verticillium longisporum* were unsuccessful, as techniques were either not sufficiently sensitive or specific enough for testing infested soils. New PCR primers, based on genome sequences for *Verticillium* species, are now available for testing.

The project has raised awareness of this ‘new disease’ and provided the first data on the distribution and importance of verticillium wilt in winter oilseed rape. The pathogen is clearly well established in some fields, particularly in eastern England and it is capable of causing yield loss in some years. There is now considerable interest from breeders and agronomists in testing varieties for resistance to verticillium wilt and this should be extended so that national resistance ratings can be produced. In the absence of a soil test, growers and agronomists should monitor crops visually for verticillium wilt and be prepared to adjust varieties and rotations where there is a threat to yield.

## 2. SUMMARY

### 2.1. Introduction/Background and aims

Verticillium wilt, caused by the soil-borne pathogen *Verticillium longisporum,* is an important disease of oilseed rape (OSR) in much of continental Europe, and of other cruciferous crops around the world. The disease was first confirmed in winter oilseed rape in two crops in the UK in Kent and Herefordshire in 2007. Its distribution and importance has not been established previously. Preliminary molecular studies have shown that isolates from these outbreaks are similar to OSR isolates from continental Europe and quite distinct from *V. dahliae* (that affects a wide range of broad-leaved plants) and from an isolate recovered from an outbreak of wilt in Brussels sprouts which occurred in the UK in the 1950s. It seems most likely that infection has been brought to the UK on seed. Further cases have been identified since the initial cases in southern and central England in 2008. As verticillium wilt causes premature ripening it may be causing loss of yield.

In Sweden, verticillium wilt can cause yield losses of up to 50%, but losses vary from year to year. There has been a recent literature review for verticillium wilt and this identified priorities for future research which this proposal addresses (Gladders, 2009).

No resistant varieties have yet been identified and no commercially viable fungicide treatments are available for field scale use, hence control currently relies mainly on extending rotations of cruciferous crops. The pathogen persists in the soil between susceptible crops as resting bodies (microsclerotia). In order to manage the disease, quantitative soil tests would be useful to quantify inoculum and inform agronomic and rotation decisions. Longer rotations on affected farms, whilst being actively debated, are not an economically attractive long-term solution, so varieties with good resistance to verticillium wilt will be required. Some sources of resistance have been identified in vegetable brassicas. The identification of markers for resistance loci would speed progress by plant breeders to improve UK varieties: ideally before the disease becomes a widespread problem.

This project aims to establish the importance of verticillium wilt in winter oilseed rape and carry out initial work on varietal resistance and the impact of agronomic factors.

*Objectives*

i) Determine the occurrence of verticillium wilt in winter oilseed rape in the UK.
ii) Investigate factors affecting disease development and yield loss under UK conditions.
iii) Support plant breeding for resistance to verticillium wilt by evaluating existing varieties under field conditions.

iv) Validate molecular diagnostic tests for confirmation of plant infection and for quantification of soil-borne inoculum.

### 2.2. Materials and methods

## 2.3.2 Determine the occurrence of verticillium wilt in winter oilseed rape

Samples of winter oilseed rape collected in late June for the CropMonitor disease survey were examined for verticillium at the University of Warwick, Wellesbourne. Twenty five plants were collected along a diagonal transect of the crop from about 100 crops each year (see www.cropmonitor.co.uk). The verticillium results were collated for all three years, together with details of the variety, the date of sowing and previous cropping.

## 3.3.2 Investigate factors affecting disease development and yield loss under UK conditions

*Soil temperature at sowing*

The influence of temperature on verticillium wilt was investigated by growing two week old seedlings of cv. Castille in 9 cm pots in growth cabinets at the University of Warwick, Wellesbourne, in infested and uninfested control compost for 6 weeks at four temperatures (7, 11, 15 and 20$^{o}$C) and then growing the plants outdoors in large pots at ADAS Boxworth until plants reached maturity. As plants started to senesce, the incidence and severity of verticillium wilt was assessed on a 0-5 index: and a 0-100 Index calculated by summation of individual plant indices and dividing by 1.25.

0 – healthy

1 = 1=25% circumference affected

2 = 26-50% circumference affected

3 =51-75% circumference affected

4 =>75% circumference affected

5 = severely affected and plant dead

Where there was early disease development there would usually be early senescence of the pods, therefore a separate score was done for early senescence.

The incidence and severity of stem canker and other pod and stem diseases was also recorded.

*Date of sowing pot experiment*

This experiment was done alongside the soil temperature experiment at ADAS Boxworth. This comprised four dates of sowing with and without verticillium inoculum and four-fold replication. Winter oilseed rape cv. Castille was sown into uninfested and infested John Innes No. 1 compost in 25cm pots (c. 30 seeds per pot) on 9 September, 5 October, 3 November and 7 December. The December sowing was done in a cool glasshouse as outdoor pots were frozen. Pots were moved outdoors when seedlings had emerged and there was no risk of severe frost. Verticillium wilt was assessed on a 0-5 index at crop maturity.

*Crop cover experiment*

This experiment was done to establish if verticillium wilt on the susceptible variety Castille was likely to be aggravated by dry conditions during flowering and seed filling. Crop covers were used to protect selected plots from rain, each with sub-plots with and without a soil incorporation of azoxystrobin (Amistar 6 l/ha) pre-drilling and four-fold replication.  The first covers were put in place on 15 April at early flowering (GS 4,1) but had suffered some damage in dry and windy conditions by 11 May. All the covers were removed by the end of flowering (23 May) and in the absence of significant rainfall, two treatments were given with 10 mm of water from a bowser. The incidence and severity of verticillium wilt was assessed on 7 July 2011 at crop maturity on 10 plants per plot using a 0-5 index. Plots were combine harvested on 25 July 2011 and yields were adjusted to 91% dry matter after moisture content determination on a Dickey John meter.

*Date of sowing and variety experiments on verticillium wilt under field conditions*

Three varieties with differing resistance to verticillium wilt were used: Castille (susceptible), Catana (resistant) and Ovation (resistant) were sown on three dates with four-fold replication. Plots were sown on 3, 20 and 30 September 2010 and 2, 14 and 28 September 2011. The crops were grown with farm inputs of fertiliser and other agrochemicals. Stem disease assessments were done close to harvest on 25 plants per plot on 14 July 2011 and 9 August 2012. Combine harvested yields were taken on 15 August 2011 and 14 August 2012. Yields were adjusted to 91% dry matter.

*Determination of yield loss from verticillium wilt*

Yield loss studies used the approach described by Gladders (2009) in HGCA Research Review No.72. Farm crops of cv. Castille were sampled at Boxworth on 8 July 2010 and 18 Jul 2012; cv. DK Cabernet was sampled at Haconby, Lincs on 14 July 2010 and cv. Castille was sampled at Haconby on 20 July 2012. Fifty individual plants were taken just prior to harvest and assessed for verticillium wilt. Plants were dried and threshed by hand and the stem diameter, number of pods, percentage dead pods, the total number of seeds, the total seed weight and the thousand seed weight was determined for each plant. The data from the crop sampled in 2008 has also been included in the overall statistical analyses.

### 2.2.3 Support plant breeding for resistance to verticillium wilt by evaluating existing varieties and novel germplasm of related crop species under glasshouse and field conditions.

The susceptibility of 24 winter oilseed rape varieties to verticilllium wilt was investigated in a field experiment sown on 28 August 2009 at Bourne, Lincs with three-fold replication. The selected varieties were sponsored by plant breeders (see Fig. 4 for variety list). Plots (30 m x 2 m) were sown at 80 seeds/m$^2$ for open pollinated varieties and 60 seeds/m$^2$ for hybrids with an Oyjord drill. Disease assessments for verticillium wilt used a 0-5 index. Where there was early senescence of the pods, a separate score was done for the percentage of the total pod canopy of each plant affected by premature ripening.  Verticillium wilt symptoms developed late in the season and assessments were made just before desiccation (14 July) and pre-harvest (26 July). The trial was combine harvested on 3 August 2010 but yield data are not presented because of bad hail damage to the trial.

### 2.2.4 Validate molecular diagnostic tests for confirmation of plant infection and for quantification of soil-borne inoculum.

This work was divided into two parts: 1) developing methodologies for extracting DNA from soil prior to PCR detection of *Verticillium* and 2) developing primers for specific detection of *V. longisporum*.

A method developed previously for extracting DNA and quantifying *V. dahliae* infecting strawberry was used for extraction of *V. longisporum* DNA from soil samples spiked with microsclerotia. A procedure for extracting *Verticillium* DNA from OSR plants was developed using young artificially inoculated plants. This method was subsequently adapted for field-grown or 8-10 week old glasshouse grown OSR plants because the vascular tissue was extremely woody.

PCR primers that were developed  to amplify both *V. dahliae* and *V. longisporum* were used to validate the above DNA extraction methods. In addition, new primers theoretically capable of distinguishing the two fungal species based previous work were also tested. These targeted a variety of gene sequences from both genomic and mitochondrial DNA.

A total of 264 soil samples for detection and quantification of *V. longisporum* were taken from various known infested fields and trial sites by ADAS.

### 2.3. Results

### 2.3.1 Determine the occurrence of verticillium wilt in winter oilseed rape

There was an average of 16% of survey crops and 3.3% of plants with verticillium wilt during 2009-2011 (Table 1). The percentage of plants affected by verticillium wilt showed wide variation with many crops showing less than 10% of plants affected, but about 5% of crops had more than 30% of plants with verticillium wilt (Table 2). It may be that the apparent increase in frequency from 2009 to 2010 may simply be due to different fields being sampled. However, in 2010, half of the infected crops had only a single infected stem (Table 2). Verticillium wilt was found as far north as Yorkshire, but it was most prevalent in parts of eastern region particularly Bedfordshire, Cambridgeshire and Northamptonshire (Fig. 1). During the project there were numerous *ad hoc* reports of verticillium wilt. These included cases from Kent and Herefordshire that were in addition to the first cases reported in 2007. These counties did not have farms with verticillium wilt on the disease survey. Thus the distribution map (Fig. 1) reflects the relative distribution of the disease, but it may occur in areas currently showing as zero on the tonal scale.

**Table 1. Occurrence of verticillium wilt in survey crops 2009-2011. Twenty five plants were assessed from each crop.**

| Year | Number of crops examined | Number of crops with verticillium | % crops affected | Mean % plants affected |
|---|---|---|---|---|
| 2009 | 95 | 10 | 10.6 | 3.0 |
| 2010 | 97 | 24 | 24.7 | 3.6 |
| 2011 | 100 | 12 | 12.0 | 3.3 |

**Table 2. The incidence of verticillium wilt (% plants affected) in individual survey crops 2009-2011. Twenty five plants were assessed from each crop.**

| Year | Number of crops in relation to categories of verticillium incidence (%plants affected) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0-5 | 6-10 | 11-15 | 16-20 | 21-30 | 31-40 | 41-50 | 51-60 | >60 |
| 2009 | 3 | 2 | 0 | 0 | 0 | 3 | 0 | 1 | 1 |
| 2010 | 12 | 2 | 4 | 1 | 2 | 0 | 1 | 2 | 0 |
| 2011 | 2 | 3 | 0 | 0 | 1 | 3 | 0 | 0 | 3 |

The factors affecting the occurrence of verticillium wilt in survey crops have been examined. The importance of specific factors was difficult to define given the relative small number of infected crops and changes in crop agronomy and weather from year to year.

A range of varieties were affected in surveyed crops, including cvs Castille, DK Cabernet, Excalibur, Vision, Ovation, Sesame and Catana. Previous cropping records for individual fields were examined for the previous year and for the previous 3 or 4 years. There were single cases of oilseed rape being grown as a second successive crop in 2009 and 2011 and this highlighted a high risk situation as 73% of plants had verticillium in the 2011 crop. Verticillium wilt was found where oilseed rape was grown after 3 or 4 year arable breaks and was not strongly linked with alternating wheat/osr rotations.

Date of sowing records ranged from early August to late September. The crops with verticillium were mainly sown in late August or early September. No verticillium wilt was found in the few crops that were sown in early August. There were some affected crops sown in mid August in 2010 and 2011, and a few late sown (after mid September) crops were affected in 2009 and 2010. There were more late sown crops in the 2009 survey reflecting the late harvest in 2008.

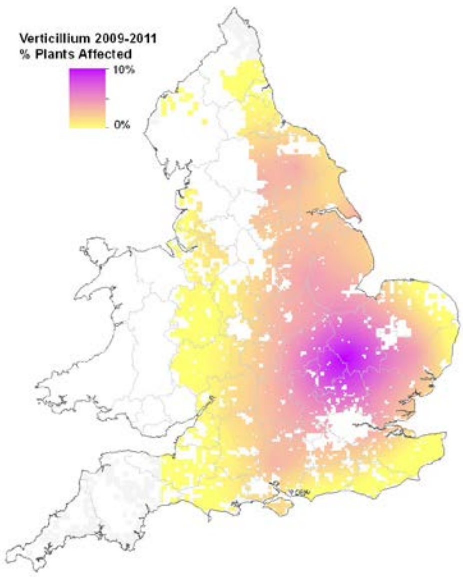


**Figure 1. Distribution of verticillium wilt in winter oilseed rape, 2009-2011.** In total, 292 crops were sampled (46 shown to be affected by verticillium wilt). During the project there were reports of verticillium wilt in Kent and Herefordshire, however, these counties did not have farms with verticillium wilt on the disease survey. Thus, the distribution map reflects the relative distribution of the disease, but it may occur in areas currently showing as zero on the tonal scale.

### 2.3.2 Investigate factors affecting disease development and yield loss under UK conditions

*Soil temperature experiment*

Temperature effects were not significant for % plant affected (range 49.7- 64.7% of plants affected in inoculated compost) or % dead pods (range 35.7-51.9 % dead pods in inoculated compost) (Fig. 2). More verticillium wilt developed where inoculated compost was used (mean 54.7% of plants affected) than with control uninoculated compost (19.3% plants affected) (P=0.001, LSD 18.3). Phoma canker was more severe at 20°C (Index 2.84) than at 15°C (Index 2.05), 11°C (Index 2.14) and 7°C (1.72). Some verticillium developed in the uninoculated controls suggesting that there have been seed-borne transmission or spread between plants during the growing season.

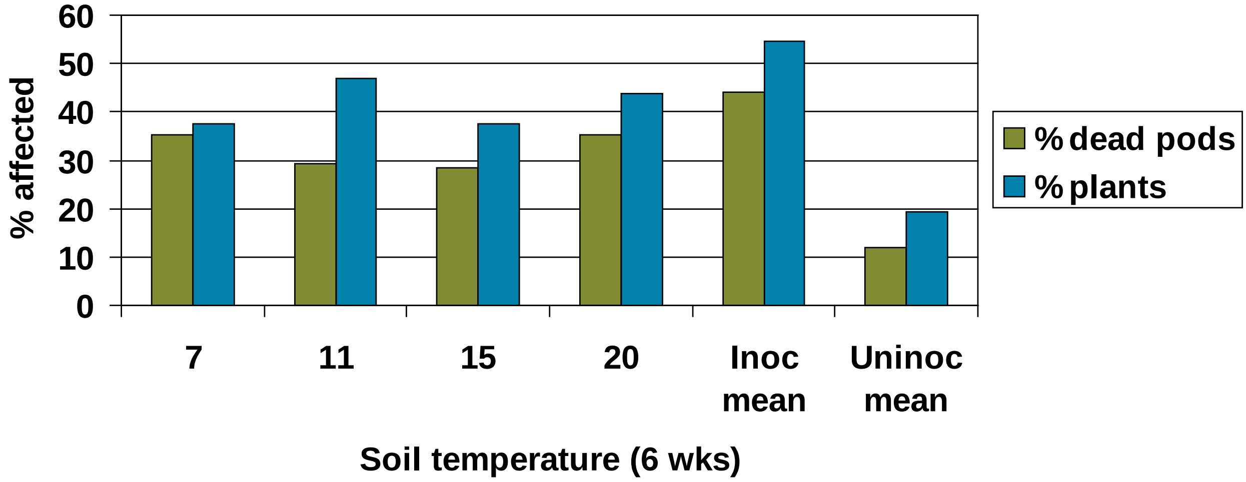


**Figure 2. Effect of different soil temperatures on the incidence (% plants) and severity (% dead pods) of verticillium wilt grown in infested compost and for inoculated versus non-inoculated compost. Young plants were grown at four temperatures (7ºC, 11ºC, 5ºC and 20ºC) in infested compost and at 20ºC in uninfested compost for 6 weeks and then grown to maturity in pots outdoors. There were no significant differences between temperatures, but inoculated compost gave more verticilium wilt (P=0.003).**

*Date of sowing pot experiment*

In the date of sowing pot experiment, there were no significant differences in verticillium incidence (mean 44% plants affected) between sowing dates (Fig. 3). Differences in severity (%dead pods) were almost significant (P=0.051, LSD would be 25.6). However, inoculation gave significantly more plants affected (44% compared with 19% in the uninoculated control; severity 26% dead pods compared with 7% in the uninoculated control). Some verticillium developed in the uninoculated controls suggesting that there have been seed-borne transmission or spread between plants during the growing season.

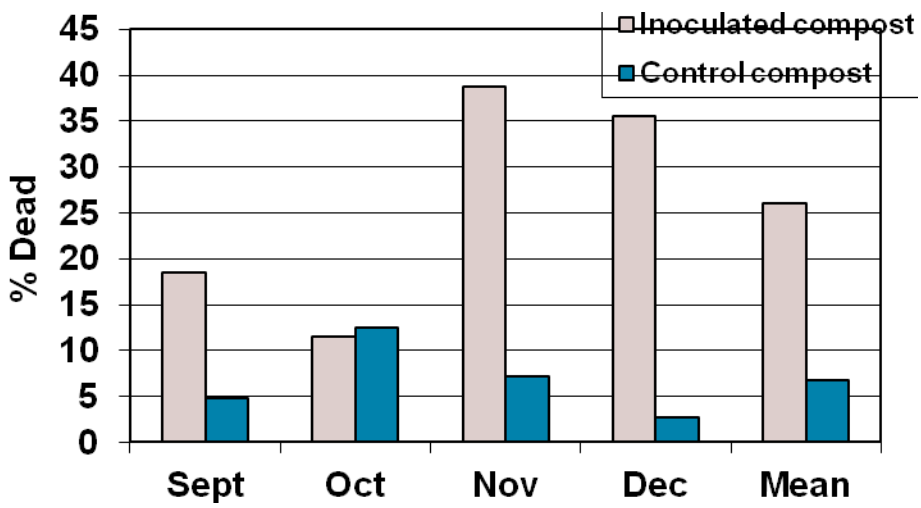


**Figure 3. Effect of date of sowing on the severity of verticillium wilt (% dead pods) in winter oilseed rape grown in pots of compost with and without inoculation with *Verticillium longisporum.* Sowing dates were 9 September, 5 October, 3 November and 7 December. The December sowing was done in a cool glasshouse as outdoor pots were frozen. Pots were moved outdoors when seedlings had emerged and there was no risk of severe frost. There was a significant effect of inoculation but not for date of sowing.**

*Crop cover experiment*

At ADAS Boxworth, the crop sown on 3 September 2011 had started to emerge by 9 September. There was no rain after 4 April and this followed a dry March with only 4.8 mm of rain. In May there were 11 days with rain and 21 mm of rain in total, most of which fell after the covers had been removed on 23 May. Crop covers did not give the anticipated differential effects because of the dry spring, so one of the treatments was changed to an application of 10 mm of water at the end of flowering. There were no significant treatment effects on verticillium wilt incidence (mean 60% of plants affected) or severity from the cover treatments or azoxystrobin. However, there was a significant yield response of 0.35 t/ha (untreated yield 3.07 t/ha) from the azoxystrobin soil treatment. There were no treatment differences for phoma canker or light leaf spot.

*Date of sowing and variety experiments on verticillium wilt under field conditions*

In Herefordshire a time of sowing experiment was done using cvs Castille (susceptible), Catana and Ovation (both considered to have some resistance to verticillium wilt). The dates of sowing,3, 20 and 30 September 2010, these gave significantly different mean yields of 5.06, 4.83 and 4.54 t/ha, respectively (LSD = 0.18) with no significant differences between the varieties. No verticillium wilt developed in the trial. Phoma canker incidence was greatest on cv. Catana and at the 20 September sowing date differences between all three varieties and all three dates of sowing were significant.

Yields in 2012 were low as the crop was severely lodged and much of the crop was flat at harvest. There were significant decreases in yield in the two later sowings and cv. Catana gave a significantly lower yield (1.60 t/ha) than cv. Castille (2.28 t/ha) and cv. Castille yielded less than cv. Ovation (2.60 t/ha). Verticillium wilt was found at low levels in the experiment at the pre-harvest assessment. Castille (11.7% of plants with verticillium) was significantly more severely affected than cvs Catana (2.7%) and Ovation (5.0%), but was not affected by date of sowing. Phoma canker was present at high levels and its incidence was greatest in the late sown treatments.

*Yield loss from verticillium wilt*

The main effect of verticillium was on thousand seed weight. A decrease in thousand seed weight was evident when verticillium was severe (index 4 or 5). The estimated yield loss at individual sites was in the range 12-24% (Table 3).

**Table 3. Summary of yield components and significant effects in relation to Verticillium stem disease index. Assessments were done on fifty individual plants that were sampled from farm crops of cv. Castille at Boxworth on 8 July 2010 and 18 Jul 2012, DK Cabernet at Haconby on 14 July 2010 and Castille at Haconby on 20 July 2012.**

| | Verticillium index | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Data | 0 | 1 | 2 | 3 | 4 | 5 | Fpr | Approx LSD |
| Stem diameter.(mm) | 14.8 | 15.0 | 15.5 | 14.6 | 15.6 | 13.8 | n.s. | 1.478 |
| Number of pods | 256.1 | 271.7 | 300.0 | 238.9 | 260.4 | 227.3 | n.s. | 64.58 |
| Total Seed Weight.(g) | 16.4 | 16.5 | 17.9 | 12.5 | 14.1 | 9.6 | 0.001 | 4.679 |
| Number of seeds | 3697 | 3340 | 3701 | 2659 | 3259 | 2411 | 0.075 | 596.2 |
| Thousand grain weight (g) | 4.6 | 5.7 | 5.8 | 5.3 | 5.0 | 4.4 | <0.001 | 0.397 |
| Seeds/pod | 14.7 | 11.9 | 12.2 | 10.5 | 11.9 | 10.1 | 0.001 | 2.830 |
| Percent dead pods | 0.1 | 12.1 | 32.9 | 51.0 | 83.9 | 98.8 | <0.001 | 3.525 |

### 2.3.3 Support plant breeding for resistance to verticillium wilt by evaluating existing varieties and novel germplasm of related crop species under glasshouse and field conditions.

*Resistance of varieties*

The replicated variety trial at Bourne, Lincs identified significant differences in the incidence of verticillium wilt between varieties (Fig. 4). Symptoms developed late in the life of the crop and the main assessment was done on the 26 July after desiccation, whilst most stems were still green. Incidence ranged from 0.7% plants affected to 24% (LSD = 10.2). The most severely affected varieties included the widely grown cvs Castille, Excalibur and Es Astrid.  , whilst varieties included Catana, Oracle, Alienor, Compass, Palace, Krypton and Cuillin had a low incidence of disease. Similar differences have been confirmed subsequently in experiments funded by plant breeders.

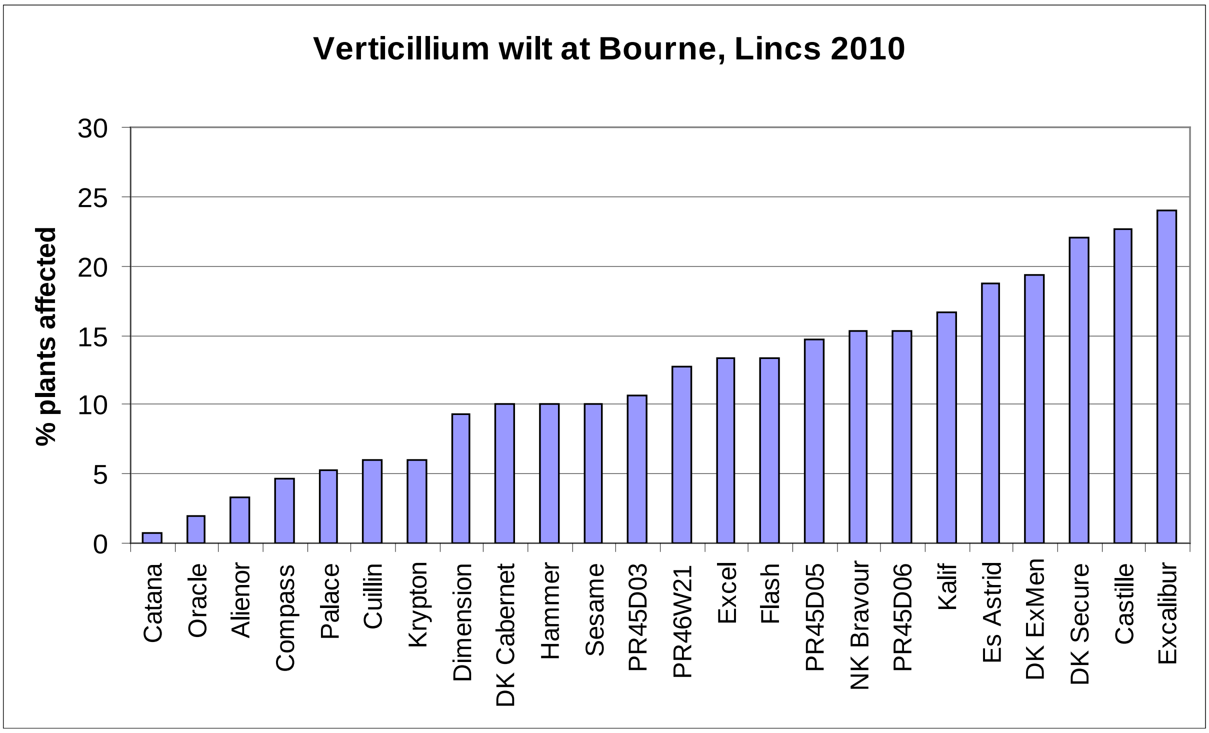


**Figure 4. Incidence of verticillium wilt in winter oilseed rape varieties at Bourne, Lincs. on 26 July 2010 (LSD = 10.2).**

### 2.4.3 Validate molecular diagnostic tests for confirmation of plant infection and for quantification of soil-borne inoculum.

Using the standard soil extraction protocol based on *V. dahliae* and the primers that could detect both *V. dahliae* and *V. longisporum,* sensitivity of detection was approx. 10-30 *V. longisporum* microsclerotia per gram in spiked soils.  This was 10 fold lower than previously found for *V. dahliae* in strawberry. This is not sensitive enough for detection of *V. longisporum* in naturally infested soils where 1-3 microsclerotia per gram can cause disease in OSR. A number of modifications of the DNA extraction method originally developed were also tested, as well as published methods, but all failed to deliver increased sensitivity. Further work is required to determine whether this low sensitivity is due to the primers being inappropriate or the *V. longisporum* microsclerotia being more refractory to DNA extraction. In contrast, good sensitivity (down to approx. 1 microsclerotium per gram) was obtained for OSR plant extracts using the same primers.

Primers designed in this project that should, theoretically, distinguish *V. longisporum* failed to reliably detect this species in soil, although at least one primer pair was specific for *V. longisporum* using DNA extracted from pure cultures. A total of 264 soil samples were tested and no *V. .longisporum* was detected in fields even where crops became heavily infected. Generally, primers either lacked reliable discrimination (especially in the presence of soil extracts) or sensitivity. In the absence of quantitative soil tests for *V. longisporum*, no data were produced for thresholds or relationships between soil microsclerotial populations and yield loss.

### 2.4. Discussion/Conclusions and implications

The project has contributed substantially to increased awareness and diagnosis of verticillium wilt, and produced the first distribution map for this disease. The average level of crop infection is still quite low although 3% plant infection is greater than that of sclerotinia stem rot (*Sclerotinia sclerotiorum*) in low epidemic years (see www.cropmonitor.co.uk). The survey did not record verticillium in Kent and Herefordshire, yet severe verticillium wilt has been recorded by ADAS in these counties, hence the distribution map should be used as a guide to the relative distribution. Agronomists and farmers should inspect crops close to harvest for signs of verticillium wilt and quantify its prevalence field by field. Future decisions on oilseed rape cropping, varieties, agronomy and rotation can then be adjusted to reduce the risk of yield loss from verticillium wilt.

There are likely to be various factors affecting the occurrence and distribution of verticillium wilt. The heavily infested areas in eastern England were amongst the first to adopt winter oilseed rape cropping in the 1970s. There are many farms using short wheat/oilseed rape rotations and plants are prone to poor rooting and drought stress. There is potential for *V. longisporum* to be introduced on seed as internal seed-borne infection or as a contaminating dust on the outside of seed. This raises concerns that seed from infected crops could be contributing to the spread of verticillium wilt. A seed test for *V. longisporum* would be a useful addition to the existing range of seed tests available to farmers.

The first variety trial produced a clear ranking of varieties for response to verticillium wilt. The broad pattern has been substantiated outside this project in more recent experiments and demonstration plots. Varieties which had a very low disease incidence in this project which could be considered ‘resistant’ are not immune to verticillium wilt and, if disease pressure is very high, could have a high incidence of infected plants and show some premature ripening. Further work is needed before firm conclusions can be made on varietal resistance and susceptibility to verticillium wilt.

The single plant studies demonstrated that plants with severe verticillium wilt (> 50% stem circumference affected) produced smaller seeds. There were several reports of verticillium causing premature ripening and loss of yield in eastern and central England in 2012, typically about 10%, but occasionally more. Further monitoring and experimentation is required to define combinations of temperature and rainfall that influence the severity of verticillium epidemics and the impact on yield. Field observations suggest that good crop agronomy can contribute to decreasing the impact of verticillium wilt and strong plants will show less premature ripening than 'stressed' plants. Where verticillium wilt is present, good crop establishment and strong rooting are likely to be beneficial. Further steps can be taken by selecting varieties with good 'resistance' to verticillium wilt before decisions need to involve longer rotations of oilseed rape (minimum of 1 year in 4).

Specific and sensitive PCR-based detection of *V. longisporum* (especially in soil) is clearly challenging and although several different sets of primers and DNA extraction methodologies were tested, none were suitable for a reliable soil or plant test. However, in a separate project, Warwick Crop Centre has generated whole genome sequence from a UK *V. longisporum* isolate. This, along with other genome sequences of *V. longisporum*, *V. dahliae* and *V. albo-atrum*, has allowed several further pairs of primers to be designed with potential specificity to *V. longisporum* mitochondrial DNA sequence. In addition, recent work by other researchers may provide the basis for designing new *V. longisporum* specific tests. Work is required to evaluate the reliability, specificity and sensitivity of these potentially useful primers.

Verticillium wilt is now an established disease in winter oilseed rape and it may affect other brassica crops including forage crops and vegetables. It is already starting to affect yield in heavily infested fields and the risk of yield of loss is likely to be greatest in eastern England where the disease is most prevalent.

### 2.4.1 Future priorities for research and knowledge transfer on verticillium wilt

- There is no published list of resistance ratings for verticillium wilt and the industry therefore lacks clear guidance for selecting varieties for infected fields. Standard protocols should be developed for determination of resistance ratings to vertcillium wilt in variety experiments.
- Further monitoring of verticillium and updated distribution maps should be produced in conjunction with CropMonitor and Defra-funded disease surveys.
- The importance of seed-borne infection should be investigated. Rapid quantitative seed test methods should be developed so that seed stocks can be tested quickly after harvest.
- The development of a soil test for verticillium wilt is still required. This is of fundamental importance for quantifying changes in soil populations in different rotations.
- Knowledge transfer to increase awareness of verticillium wilt and how it can be managed should continue to be supported. There is still time for decisions on farms to influence the build up and impact of this disease.

## 3. TECHNICAL DETAIL

### 3.1. Introduction

Verticillium wilt caused by the soil-borne pathogen *Verticillium longisporum* is an important disease of oilseed rape (OSR) in much of continental Europe, and of other cruciferous crops around the world (Eastburn and Paul, 2007**;** Karapapa, Bainbridge and Heale, 1997; Collins, Okoli, Morton, Parry, Edwards and Barbara, 2003). The disease was first confirmed in winter oilseed rape in two crops in the UK in Kent and Herefordshire in 2007 (Gladders, Smith, Kirkpatrick, Clewes, Grant, Barbara, Barnes and Lane, 2011) but its distribution and importance has not been established. Preliminary molecular studies have shown that isolates from these outbreaks are similar to OSR isolates from continental Europe (D Barbara, unpublished data; Collins et al., 2003) and quite distinct from *V. dahliae* (that affects a wide range of broad-leaved plants) and from an isolate recovered from an outbreak of wilt in Brussels sprouts which occurred in the UK in the 1950s (Isaac, 1957). It seems most likely that infection has been brought to the UK on seed (Svensson and Lerennius, 1987). Further cases have been identified since the initial cases in southern and central England in 2008. The disease has recurred in 2008 in different fields on the two farms affected in 2007 and new cases with severe premature ripening caused by verticillium wilt have been confirmed in 2008. Between 1% and 90% of plants have shown symptoms in affected fields (ADAS unpublished data). As verticillium wilt is causing premature ripening it may be causing loss of yield.

Reports for arable consultants also suggest that verticillium wilt appears to be established in England with potential to cause significant loss of yield if it is not managed effectively. In Sweden, verticillium wilt can cause yield losses of up to 50%, but losses vary from year to year. In the UK, individual plants severely affected by verticillium wilt showed a 60% yield loss (Gladders, 2009). As verticillium wilt causes premature ripening, it may be one of the factors contributing to static or declining oilseed rape yields. There has been a recent literature review for verticillium wilt and this identified priorities for future research (Gladders, 2009) which the work described here addresses.

No resistant varieties have yet been identified and no commercially viable fungicide treatments are available for field scale use (Eastburn and Paul, 2007), hence control currently relies mainly on extending rotations of cruciferous crops (Kreye, Steinbach and Wolf, 2006; Eynck, Koopmann, Grunewaldt-Stoecker, Karlovsky and von Tiedemann, 2007). The pathogen persists in the soil between susceptible crops as resting bodies (microsclerotia). In order to manage the disease, quantitative soil tests are required to quantify inoculum and inform agronomic and rotation decisions. However, longer rotations on affected farms, whilst being actively debated (Stobart, 2009), are not an economically attractive solution for the future, so varieties with good resistance to verticillium wilt will be required. Some sources of resistance have been identified in *Brassica oleracea* (Rygulla, Snowdon, Eynck, Koopmann, von Tiedemann, Lüchs and Friedt, 2007). The identification of markers for resistance loci would speed progress by plant breeders to improve UK varieties: ideally before the disease becomes a widespread problem.

This project aims to establish the importance of verticillium wilt in winter oilseed rape and carry out initial work on varietal resistance and the impact of agronomic factors.

#### 3.1.1 Objectives

i) Determine the occurrence of verticillium wilt in winter oilseed rape in the UK.
ii) Investigate factors affecting disease development and yield loss under UK conditions.
iii) Support plant breeding for resistance to verticillium wilt by evaluating existing varieties and novel germplasm of related crop species under glasshouse and field conditions.
iv) Validate molecular diagnostic tests for confirmation of plant infection and for quantification of soil-borne inoculum.

### 3.2. Materials and methods

#### 3.2.1 Determine the occurrence of verticillium wilt in winter oilseed rape

Samples of winter oilseed rape collected in late June for the CropMonitor disease survey were used to provide an objective assessment of the incidence of verticillium wilt. Sites are selected from June Census returns and stratified by farm size within each region according the area of arable cropping in each region. Twenty five plants are collected along a diagonal transect of the crop from about 100 crops each year (see www.cropmonitor.co.uk) and assessed for stem and pod diseases by FERA at York. For this project, the lower part of the stem and roots were retained and air-dried after the initial general disease assessment in harvest years 2009, 2010 and 2011. The dried stems were then sent to the University of Warwick, Wellesbourne, where detailed examination for verticillium was carried out. The woody stems and roots were difficult to subsample and test using DNA extraction techniques. After preliminary examination of 24 samples from the 2010 survey samples, the favoured technique was to split the stems and roots in half longitudinally and incubate these in damp chambers for several days. Visual and microscopic examination on each stem was then used to identify *V. longisporum* by microsclerotia and sporulating fungal growth. Identifications were also checked using PCR tests.

The verticillium results were collated for all three years together with details of the variety, the date of sowing and previous cropping. The distribution was mapped using a simple polynomial interpolation method. The smoothed out map generalises the picture taking into account all the zeroes and the density of the points, so that isolated points contribute less.

### 3.2.2 Investigate factors affecting disease development and yield loss under UK conditions

*Effect of soil temperature at sowing*

The influence of temperature on verticillium wilt was investigated by growing two week old seedlings cv. Castille in 9 cm pots in growth cabinets at the University of Warwick, Wellesbourne in infested and uninfested control compost for 6 weeks at four temperatures (7, 11, 15 and 20$^{o}$C) (Table 1) and then growing the plants outdoors in large pots at ADAS Boxworth until plants reached maturity. There were 20 plants in infested compost and 12 plants in non-infested compost at each temperature. These were transplanted into 25 cm pots containing commercial John Innes No. 1 compost (4 plants spaced equidistant per pot) and grown to maturity at ADAS Boxworth with minimal inputs of fertiliser and pesticides to minimise interference with verticillium development. The infested soil was a John Innes compost containing microsclerotia derived from the equivalent of one short piece of oilseed rape stubble per 25 cm pot.

**Table 1. Treatments in the soil temperatures and compost infestation experiment 2010/11.**

| Trt | Target temperature (ºC) | Compost treatment in John Innes No. 1 |
|---|---|---|
| 1 | 7 | Infested with *V. longisporum* |
| 2 | 7 | Un-infested |
| 3 | 11 | Infested with *V. longisporum* |
| 4 | 11 | Un-infested |
| 5 | 15 | Infested with *V. longisporum* |
| 6 | 15 | Un-infested |
| 7 | 20 | Infested with *V. longisporum* |
| 8 | 20 | Un-infested |

As plants started to senesce, the incidence and severity of verticillium wilt was assessed on all the plants using a 0-5 stem colonisation index (see below) and recording the percentage of prematurely ripened or dead pods.

Stem colonisation was assessed on a 0-5 index:

0 – healthy

1 = 1=25% circumference affected

2 = 26-50% circumference affected

3 =51-75% circumference affected

4 =>75% circumference affected

5 = severely affected and plant dead

Disease severity was expressed as 0-100 index calculated by summation of individual plant indices and dividing by 1.25. Where there was early disease development there would usually be early senescence of the pods. A separate score was required for early senescence if this did not follow the circumference index scoring.

The incidence and severity of stem canker and other pod and stem diseases was also recorded.

*Date of sowing pot experiment*

This experiment was done alongside the soil temperature experiment at ADAS Boxworth. The factorial design comprised four dates of sowing with and without verticillium inoculum and four-fold replication (Table 2). Winter oilseed rape cv. Castille was sown into uninfested and infested John Innes No. 1 compost in 25cm pots (c. 30 seeds per pot) on 9 September, 5 October, 3 November and 7 December. The December sowing was done in a cool glasshouse as outdoor pots were frozen. Pots were moved outdoors when seedlings had emerged and there was no risk of severe frost. Disease assessments for verticillium wilt were done as described for the Soil Temperature experiment.

**Table 2. Treatments in the dates of sowing and compost infestation experiment 2010/11.**

| Trt | Target date of sowing | Compost treatment in John Innes No. 1 |
|---|---|---|
| 1 | Early September | Infested with *V. longisporum* |
| 2 | Early September | Un-infested |
| 3 | Early October | Infested with *V. longisporum* |
| 4 | Early October | Un-infested |
| 5 | Early November | Infested with *V. longisporum* |
| 6 | Early November | Un-infested |
| 7 | Early December | Infested with *V. longisporum* |
| 8 | Early December | Un-infested |

*Crop cover experiment*

This experiment was done to establish if verticillium wilt on the susceptible variety Castille was likely to be aggravated by dry conditions during flowering and seed filling. Crop covers were used to protect selected plots from rain and allow comparison of the effects of ‘normal’ and ‘dry’ conditions on disease severity and yield (Fig. 1). There were four crop cover treatments in the original design, each with sub-plots, with and without a soil incorporation of azoxystrobin (Amistar) pre-drilling and four-fold replication (Table 3). Azoxystrobin (as 6 l/ha of Amistar) was applied to soil in 500 l/ha of water and incorporated by power harrow to a depth of 7.5cm on 3 September 2010 prior to sowing on the same day. Plots (12.0 m x 1.68 m) were sown with cv. Castille at 80 seeds/$m^2$, using an Oyjord drill, at ADAS Boxworth in a field that had shown low levels of verticillium wilt in winter oilseed rape in 2008. Additional discard plots were sown to allow covers to extend beyond the individual experimental plots.

The metal framework for the polythene crop covers was erected prior to flowering and polythene sheeting positioned over the treated blocks. The crop cover dates allowed one set of covers to be moved from the early cover treatment to the late cover treatment. The first covers were put in place on 15 April at early flowering (GS 4,1) but suffered some damage in windy conditions by 11 May. All the covers were removed by the end of flowering (23 May) and in the absence of significant rainfall, treatments 7 and 8 were given 10 mm of water by hose from a bowser (Table 3).

The plots were grown with farm inputs of fertiliser and pesticides to minimise non-target disease effects. A spray of flusilazole (as Sanction 0.4 l/ha) was applied on 30 January for control of phoma leaf spot and stem canker.

The incidence and severity of verticillium wilt was assessed on 7 July 2011 at crop maturity on 10 plants per plot using the 0-5 index described for the pot experiments. Plots were combine harvested on 25 July 2011, yields were adjusted to 91% dry matter after moisture content determination on a Dickey John meter.

**Figure 1. Field view of crop covers experiment at Boxworth 2011. Photograph taken 16th April 2011.**

**Table 3. Treatments in crop cover and soil treatment experiment 2010/11.**

| Trt | Target crop cover treatment | Actual crop cover treatment | Soil treatment on sub-plot |
|---|---|---|---|
| 1 | Uncovered | Uncovered | Nil |
| 2 | Uncovered | Uncovered | Amistar* |
| 3 | Covered late March to end June | Covered at flowering | Nil |
| 4 | Covered late March to end June | Covered at flowering | Amistar |
| 5 | Covered late March to end May | Covered at flowering | Nil |
| 6 | Covered late March to end May | Covered at flowering | Amistar |
| 7 | Covered late May to end June | Uncovered with 10 mm water applied 25 May | Nil |
| 8 | Covered late May to end June | Uncovered with 10 mm water applied 25 May | Amistar |

* Amistar (6l/ha in 500 l water/ha) applied to soil after initial cultivations then cultivated into the seedbed 7.5 cm deep and crop sown as soon as possible thereafter.

*Date of sowing and variety experiments on verticillium wilt under field conditions*

Three varieties with differing resistance to verticillium wilt were used: Castille (susceptible), Catana (resistant) and Ovation (resistant). Seed of cvs Castille and Catana was obtained from commercial sources and seed of cv. Ovation was provided by Frontier. The experiment had a factorial design with three varieties and three dates of sowing and four-fold replication. The treatments were blocked by date of sowing to facilitate drilling on different dates. The actual dates of sowing achieved were rather later than the targets because of weather and ground conditions. Plots (18 m x 3.5m) were sown at 100 seeds/m$^2$ and 70 seeds/m$^2$ in 2012 by plot drill on the dates indicated in Table 4. Plant counts were done on all plots at 7-14 day intervals from early October until November. The crops were grown with farm inputs of fertiliser and other agrochemicals. Stem disease assessments were done close to harvest on 25 plants per plot on 14 July and 9 August 2012. Combine harvested yields were taken on 15 August 2011 and 14 August 2012. Yields were adjusted to 91% dry matter.

**Table 4. Cultivars and dates of sowing 2010/11 and 2011/12.**

| Trt | Cultivar | Target date of sowing | Actual dates of sowing 2010/11 | Actual dates of sowing 2011/12 |
|---|---|---|---|---|
| 1 | Castille | 20 August | 3 September | 2 September |
| 2 | Catana | 20 August | 3 September | 2 September |
| 3 | Ovation | 20 August | 3 September | 2 September |
| 4 | Castille | Early September | 20 September | 14 September |
| 5 | Catana | Early September | 20 September | 14 September |
| 6 | Ovation | Early September | 20 September | 14 September |
| 7 | Castille | Mid September | 30 September | 28 September |
| 8 | Catana | Mid September | 30 September | 28 September |
| 9 | Ovation | Mid September | 30 September | 28 September |

*Determination of yield loss from verticillium wilt*

Yield loss studies used the approach described by Gladders (2009) in HGCA Research Review No. 72. Farm crops of cv. Castille were sampled at Boxworth on 8 July 2010 and 18 Jul 2012; cv. DK Cabernet was sampled at Haconby, Lincs on 14 July 2010 and unused plots of cv. Castille were sampled at Haconby in 20 July 2012. In all these crops, the seed was ripe in the most severely affected plants and brown on the other plants (GS 6,5-6,7). No crops were sampled in 2011 as suitable crops with severe verticillium wilt were not found. Fifty individual plants were taken just prior to harvest from heavily infested crops, assessed for verticillium wilt(as previously described) and had their diameter at the stem base recorded. The selected plants were of similar stem size but exhibited a range of disease severities from healthy to dead. The percentage of dead pods was recorded.

After plants had been allowed to dry in paper sacks or bags, the plants were threshed by hand and the number of pods, the total number of seeds, the total seed weight and the thousand seed weight was determined for each plant. The relationships between yield components and verticillium scores were then examined using correlation matrices, regression analyses and various biplots to define their contribution to yield. The data were analysed using the disease indices (0-5 scale) and, where appropriate back converting the indices to percentage stem circumference affected. .Note that as the verticillium indices are categorical values, interpolated values were calculated to enable regression lines to be fitted to the data (i.e. index 1 is for lesions varying from 1-25% stem circumference affected so the interpolated value is mid-range = 12.5%). The data from the crop sampled in 2008 (Gladders, 2009) was included in the overall analyses.

### 3.2.3 Support plant breeding for resistance to verticillium wilt by evaluating existing varieties and novel germplasm of related crop species under glasshouse and field conditions.

The susceptibility of 24 winter oilseed rape varieties to verticilllium wilt was investigated in a field experiment sown on 28 August 2009 at Bourne, Lincs with three-fold replication. The selected varieties were sponsored by plant breeders (see Table 34 and Fig. 10 for variety list). Plots (30 m x 2 m) were sown at 80 seeds/$m^2$ for open pollinated varieties and at 60 seeds/$m^2$ for hybrids with an Oyjord drill. The crop received farm inputs of fertiliser and pesticides, except that plant growth regulatory fungicides (e.g. tebuconazole) were not applied in spring. Fungicides were applied to control phoma leaf spot and light leaf spot in autumn and winter, and at flowering for sclerotinia control.

Verticillium wilt symptoms, assessed as previously described, developed late in the season and assessments were made just before desiccation (14 July) and pre-harvest (26 July). The first assessment was done when seeds ranged from green-brown to brown-black (GS 6,4-6,6). There were few plants with clear external symptoms and all plants (25/plot at the ‘field’ end of the trial) were examined internally in transverse sections. There was only slight vascular discoloration in some plants and overall incidence was low at 7.8% plants affected. This did not give significant differences between varieties and a second assessment was done on 26 July on samples of 25 plants at each end of the plots. The disease data was analysed separately and as a combined dataset (50 plants/plot).

The trial field was badly damaged by a hailstorm soon after the final disease assessment and this caused considerable loss of seed from the crop. The trial was combine harvested, but yield data are not presented because of the hail damage.

#### 3.2.4 Validate molecular diagnostic tests for confirmation of plant infection and for quantification of soil-borne inoculum.

This work was divided into two parts: 1) developing methodologies for extracting DNA from soil prior to PCR detection of *Verticillium* and 2) developing primers for specific detection of *V. longisporum*.

A method based on the PowerSoil DNA Isolation Kit (Cambio Ltd) developed previously at Wellesbourne for extracting DNA and quantifying *V. dahliae* infecting strawberry (Krishnamurthy, PhD thesis, Aberdeen University, 2004) was used for extraction of *V. longisporum* DNA from soil samples spiked with microsclerotia. A procedure for extracting *Verticillium* DNA from young artificially inoculated oilseed rape plants was also developed based on the DNeasy Plant Mini Kit (Qiagen Ltd) followed by a clean-up though a PVP column. This method was subsequently adapted for field-grown or 8-10 week old glasshouse grown oilseed rape plants, because the vascular tissue was extremely woody. A new first stage was added to the extraction procedure which involved cutting short sections of the hypocotyls (for older glasshouse plants) or from stem bases (for field plants) and removing the superficial tissues. The exposed vascular cylinders were then ground on sandpaper and DNA extracted from the ground tissue using a Fastprep macerator and the DNeasy Plant Mini Kit, with a final clean-up of the extract using a PVP spin-column.

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PCR primers developed by Dez Barbara to amplify both *V. dahliae* and *V. longisporum* were used to validate the above DNA extraction methods (DB19F – CGACTTCGTCCCGAGCTCTAG; DB22R-CTCGTGGGACTACTGTTCCA). New primers theoretically capable of distinguishing the two fungal species based on Dez Barbara's previous work and published information were also tested using DNA samples from pure cultures, soil spiked with microsclerotia of *V. longisporum* and from the field (Appendix 3.6.5, Table 38). These targeted a variety of gene sequences from both genomic and mitochondrial DNA.

Th soil samples for detection and quantification of *V. longisporum* were taken from various known infested fields and trial sites by ADAS. A total of 264 samples were tested. Many of the fields had sampling points defined by GPS so that samples could be taken at the same locations in future years.

## 3.3 Results

### 3.3.1 Determine the occurrence of verticillium wilt in winter oilseed rape

Initial assessment of the CropMonitor disease survey samples gave no positive detection (for 24 samples collected in June 2010) and so the procedure was modified to include incubation. Testing of further samples from 2010 gave 24 positives from 73 samples. All these 97 samples have been included in the results presented for 2010 and used in comparison with results from 2009 and 2011 (Table 5). There was an average of 16% of survey crops and 3.3% of plants with verticillium wilt during 2009-2011. The percentage of plants affected by verticillium wilt showed wide variation with many crops showing less than 10% of plants affected but about 5% of crops had more than 30% of plants with verticillium wilt (Table 6).

The apparent increase in frequency from 2009 to 2010 may simply be due to different fields being sampled. However, of the 2010 samples which were positive, 12 (50% of those samples with any infection) had only a single infected stem detected (infection was detected in more than 50% of stems in a few fields, suggesting long-established infection in those fields). The lowest incidence of infected stems for 2009 positive samples was 8% and in the worst affected samples more 50% of the stems were infected. This higher proportion of very low level infections in 2010 might suggest that new fields are becoming infected, but as yet the sample numbers are too low to be certain.

The distribution of affected crops is shown in Figure 2. This should be regarded as a provisional representation of verticillium wilt, given that only 46 survey crops had verticillium wilt and only 292 crops were sampled. The number of crops sampled in individual counties was low and a finer scale representation of the distribution is not appropriate at present. Verticillium wilt was found as far north as Yorkshire, but it was most prevalent in parts of eastern region (the darker tones shown in Fig. 2), particularly Bedfordshire, Cambridgeshire and Northamptonshire.

**Table 5. Occurrence of verticillium wilt in survey crops 2009-2011. Approximately twenty five plants were assessed from each crop.**

| Year | Number of crops samples examined | Number of crops with Verticillium | % crops affected | Mean % plants affected |
|---|---|---|---|---|
| 2009 | 95 | 10 | 10.6 | 3.0 |
| 2010 | 97 | 24 | 24.7 | 3.6 |
| 2011 | 100 | 12 | 12.0 | 3.3 |

**Table 6. The incidence of verticillium wilt (% plants affected) in individual survey crops 2009-2011. Approximately twenty five plants were assessed from each crop.**

| Year | Number of crops in relation to categories of verticillium incidence (%plants affected) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0-5 | 6-10 | 11-15 | 16-20 | 21-30 | 31-40 | 41-50 | 51-60 | >60 |
| 2009 | 3 | 2 | 0 | 0 | 0 | 3 | 0 | 1 | 1 |
| 2010 | 12 | 2 | 4 | 1 | 2 | 0 | 1 | 2 | 0 |
| 2011 | 2 | 3 | 0 | 0 | 1 | 3 | 0 | 0 | 3 |

During the project there were numerous *ad hoc* reports of verticillium wilt from farmers and agronomists and records from various experimental sites. These included cases from Kent and Herefordshire that were in addition to the first cases reported in 2007. These counties did not have farms with verticillium wilt detected on the disease survey. Thus the distribution map reflects the relative distribution of the disease, but it may occur in areas currently showing as zero on the tonal scale.

The factors affecting the occurrence of verticillium wilt in survey crops have been examined (Table 7). The importance of specific factors is difficult to define given the relative small number of infected crops and changes in crop agronomy and weather from year to year. In 2009, verticillium was found in three cvs Castille, DK Cabernet and Ovation but not in widely grown varieties such as cvs Es Astrid and Excalibur. The proportion of the crop in cv. Castille declined between 2009 and 2010, whilst the proportion of cv. DK Cabernet increased. In 2010, cvs Castille, DK Cabernet and Ovation were again affected by verticillium wilt and there were records on cvs Excalibur, Vision and Catana. In 2011, cvs Castille and DK Cabernet were affected for a third successive year, cv. Excalibur for a second season and there were the first cases on cv. Sesame. There were no records on cvs Vision or Catana in 2011.

Previous cropping records for individual fields were examined for the previous year (Table 8) and for the previous 3 or 4 years (Table 9). Most winter oilseed rape crops surveyed were grown after winter wheat and most of the others followed another cereal. Most verticillium wilt was found after winter wheat which is the predominant cereal crop, particularly in eastern England. There were single cases of oilseed rape being grown as a second successive crop in 2009 and 2011 and this highlighted a high risk situation as 73% of plants had verticillium in the 2011 crop. Verticillium wilt was also found in one winter oilseed rape after grass in 2010. The presence of grass cropping suggests a less intensive arable rotation, yet there was still verticillium wilt in this situation. The previous oilseed rape cropping history did not show a strong link with alternating wheat/osr rotations. Verticillium may become more prevalent in short rotations in future as soil inoculum increases more rapidly.

Date of sowing records ranged from early August to late September (Table 10). The crops with verticillium were mainly sown in late August or early September. No verticillium wilt was found in the few crops that were sown in early August. There were some affected crops sown in mid August in 2010 and 2011 and a few late sown (after mid September) crops were affected in 2009 and 2010. There were more late sown crops in the 2009 survey reflecting the late harvest in 2008.

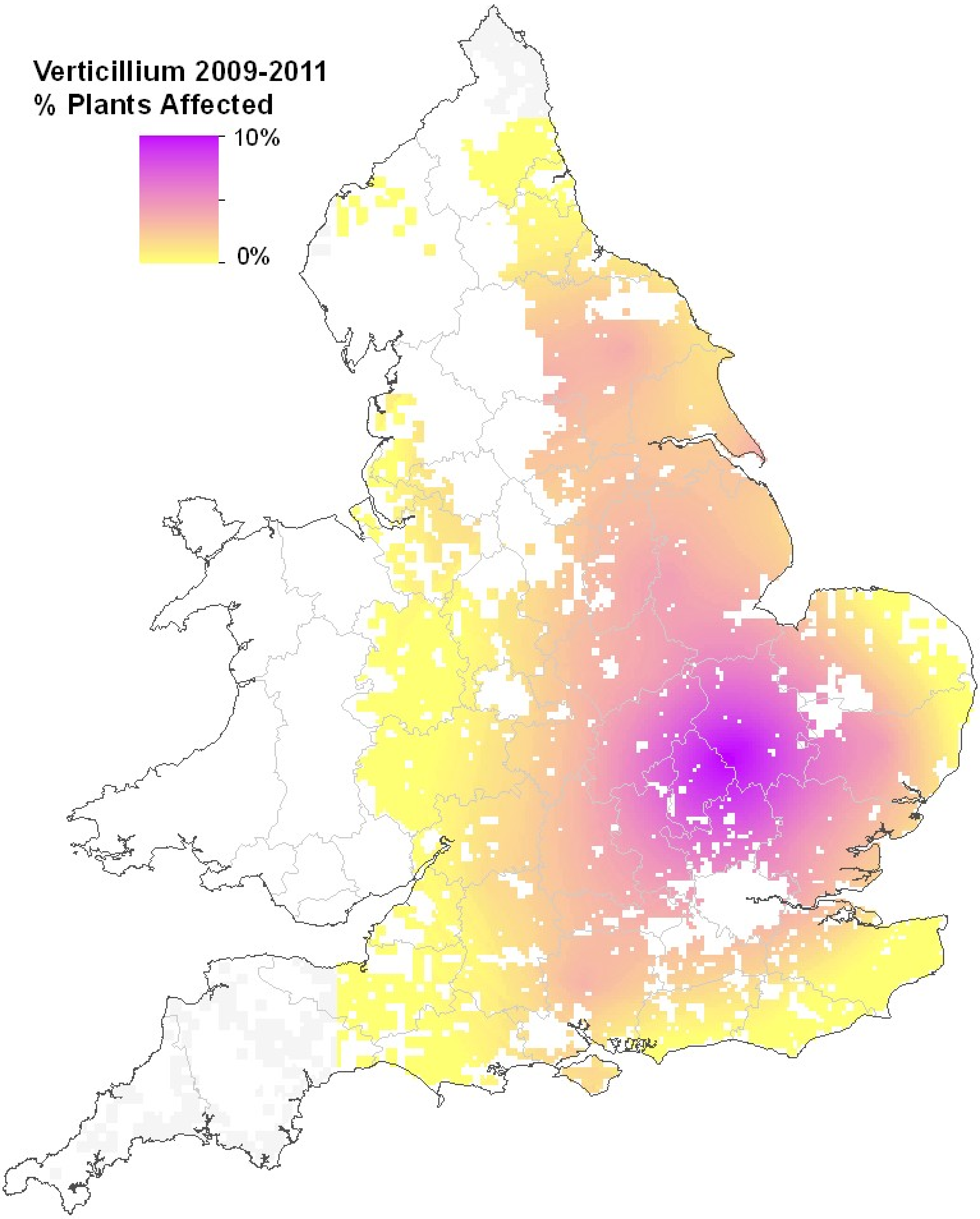


**Figure 2. Distribution of verticillium wilt in winter oilseed rape, 2009-2011.** In total, 292 crops were sampled (46 shown to be affected by verticillium wilt). During the project there were reports of verticillium wilt in Kent and Herefordshire, however, these counties did not have farms with verticillium wilt on the disease survey. Thus the distribution map reflects the relative distribution of the disease, but it may occur in areas currently showing as zero on the tonal scale.

**Table 7. Incidence of verticillium wilt in relation to variety of winter oilseed rape 2009 (a), 2010 (b) and 2011 (c). Twenty five plants were assessed from each crop.**

**a)**

| 2009 | | | |
|---|---|---|---|
| Variety | No. crops sampled | % crops affected | % plants affected |
| Castille | 33 | 15.2 | 5.1 |
| ES Astrid | 13 | 0.0 | 0.0 |
| Excalibur | 11 | 0.0 | 0.0 |
| DK Cabernet | 5 | 20.0 | 11.7 |
| Ovation | 5 | 40.0 | 7.8 |
| Flash | 2 | 0.0 | 0.0 |
| Catana | 3 | 0.0 | 0.0 |
| Marcant | 2 | 0.0 | 0.0 |
| Others | 21 | - | - |
| National | 95 | 10.6 | 3.0 |

**b)**

| 2010 | | | |
|---|---|---|---|
| Variety | No. crops sampled | % crops affected | % plants affected |
| Castille | 18 | 22.2 | 2.0 |
| DK Cabernet | 17 | 47.1 | 3.4 |
| Excalibur | 10 | 40.0 | 7.4 |
| Dimension | 8 | 0.0 | 0.0 |
| Vision | 8 | 25.0 | 3.8 |
| Catana | 7 | 14.3 | 6.5 |
| Ovation | 5 | 40.0 | 6.9 |
| Flash | 3 | 0.0 | 0.0 |
| Others | 21 | - | - |
| National | 97 | 24.7 | 3.6 |

**c)**

| 2011 | | | |
|---|---|---|---|
| Variety | No. crops sampled | % crops affected | % plants affected |
| DK Cabernet | 24 | 16.7 | 5.2 |
| Excalibur | 12 | 8.3 | 1.4 |
| Castille | 8 | 37.5 | 10.5 |
| Vision | 8 | 0.0 | 0.0 |
| Sesame | 6 | 33.3 | 15.8 |
| Catana | 5 | 0.0 | 0.0 |
| Palmedor | 4 | 0.0 | 0.0 |
| Dimension | 4 | 0.0 | 0.0 |
| DK Expower | 3 | 0.0 | 0.0 |
| Others | 26 | - | - |
| National | 100 | 12.0 | 3.3 |

**Table 8. Effect of previous cropping on the incidence of verticillium wilt 2009 (a), 2010 (b) and 2011 (c). Twenty five plants were assessed from each crop.**

**a)**

| 2009 | | | |
|---|---|---|---|
| Previous crop | No. crops sampled | % crops affected | % plants affected |
| Winter wheat | 56 | 10.7 | 3.5 |
| Other cereals | 35 | 8.6 | 2.3 |
| Beans/peas | 0 | - | - |
| Potatoes | 0 | - | - |
| Grass | 1 | 0.0 | 0.0 |
| Fallow | 1 | 0.0 | 0.0 |
| Other | 0 | - | - |
| Oilseed rape | 1 | 100.0 | 4.3 |
| Set aside | 0 | - | - |
| Unknown | 1 | 0.0 | 0.0 |
| National | 95 | 10.6 | 2.9 |

**b)**

| 2010 | | | |
|---|---|---|---|
| Previous crop | No. crops sampled | % crops affected | % plants affected |
| Winter wheat | 55 | 30.9 | 4.5 |
| Other cereals | 38 | 15.8 | 2.5 |
| Beans/peas | 0 | - | - |
| Potatoes | 0 | - | - |
| Grass | 1 | 100.0 | 4.2 |
| Fallow | 1 | 0.0 | 0.0 |
| Other | 0 | - | - |
| Oilseed rape | 0 | - | - |
| Set aside | 1 | 0.0 | 0.0 |
| Unknown | 1 | 0.0 | 0.0 |
| National | 97 | 24.7 | 3.6 |

**c)**

| 2011 | | | |
|---|---|---|---|
| Previous crop | No. crops sampled | % crops affected | % plants affected |
| Winter wheat | 61 | 9.8 | 3.3 |
| Other cereals | 36 | 13.9 | 1.6 |
| Beans/peas | 0 | - | - |
| Potatoes | 0 | - | - |
| Grass | 1 | 0.0 | 0.0 |
| Fallow | 0 | - | - |
| Other | 0 | - | - |
| Oilseed rape | 1 | 100.0 | 73.1 |
| Set aside | 0 | - | - |
| Unknown | 1 | 0.0 | 0.0 |
| National | 100 | 12.0 | 3.3 |

**Table 9. Effect of previous oilseed rape cropping on the incidence of verticillium wilt 2009 (a), 2010 (b) and2011 (c). Twenty five plants were assessed from each crop.**

**a)**

| 2009 | | | |
|---|---|---|---|
| Previous crop | No. crops sampled | % crops affected | % plants affected |
| Oilseed rape | 1 | 100.0 | 4.3 |
| Wheat/OSR/wheat | 8 | 0.0 | 0.0 |
| Wheat/wheat/OSR | 19 | 15.8 | 8.1 |
| Other cereal/wheat/OSR | 7 | 14.3 | 1.2 |
| Other cereal/other cereal/OSR | 0 | 0.0 | 0.0 |
| Three previous non OSR crops | 13 | 30.8 | * |
| Four or more previous non OSR crops | 44 | 2.3 | * |
| Other / unknown info | 3 | 0.0 | 0.0 |
| National | 95 | 10.6 | 2.9 |

**b)**

| 2010 | | | |
|---|---|---|---|
| Previous crop | No. crops sampled | % crops affected | % plants affected |
| Oilseed rape | 0 | 0.0 | 0.0 |
| Wheat/OSR/wheat | 5 | 40.0 | 3.1 |
| Wheat/wheat/OSR | 13 | 30.8 | 2.3 |
| Other cereal/wheat/OSR | 9 | 11.1 | 1.0 |
| Other cereal/other cereal/OSR | 0 | 0.0 | 0.0 |
| Three previous non OSR crops | 22 | 18.2 | * |
| Four or more previous non OSR crops | 42 | 26.2 | * |
| Other / unknown info | 6 | 33.3 | |
| National | 97 | 24.7 | 3.6 |

**c)**

| 2011 | | | |
|---|---|---|---|
| Previous crop | No. crops sampled | % crops affected | % plants affected |
| Oilseed rape | 1 | 100.0 | 73.1 |
| Wheat/OSR/wheat | 9 | 11.1 | 0.9 |
| Wheat/wheat/OSR | 18 | 16.7 | 7.5 |
| Other cereal/wheat/OSR | 11 | 18.2 | 3.6 |
| Other cereal/other cereal/OSR | 0 | 0.0 | 0.0 |
| Three previous non OSR crops | 18 | 22.2 | * |
| Four or more previous non OSR crops | 41 | 2.4 | * |
| Other / unknown info | 2 | 0.0 | 0.0 |
| Set aside | 0 | | |
| Unknown | 1 | 0.0 | 0.0 |
| National | 100 | 12.0 | 3.3 |

**Table 10. Effect of sowing date on the occurrence of verticillium wilt 2009-2011**

| | 2009 | | 2010 | | 2011 | |
|---|---|---|---|---|---|---|
| Date | No. crops sampled | % crops affected | No. crops sampled | % crops affected | No. crops sampled | % crops affected |
| Before 10th August | 0 | 0.0 | 5 | 0.0 | 3 | 0.0 |
| 10 - 19 August | 1 | 0.0 | 9 | 4.2 | 17 | 2.0 |
| 20 - 31 August | 35 | 6.3 | 54 | 9.4 | 42 | 4.0 |
| 1 - 14 September | 26 | 2.1 | 22 | 8.3 | 34 | 6.1 |
| After 15th September | 31 | 2.1 | 4 | 1.0 | 2 | 0.0 |
| National | 93 | 10.6 | 94 | 25.0 | 98 | 12.1 |

### 3.3.2 Investigate factors affecting disease development and yield loss under UK conditions

*Soil temperature experiment*

More verticillium wilt was identified where inoculated compost was used (mean 54.7% of plants affected) than in the control uninoculated compost (19.3% plants affected; P=0.001; LSD 18.3). Temperature effects were not significant for percentage plant affected (range 49.7- 64.7% of plants affected in inoculated compost ) or percentage dead pods (range 35.7-51.9 % dead pods in inoculated compost ) (Fig. 3).

The influence of temperature on verticillium wilt was investigated by growing young seedlings cv. Castille in growth cabinets in infested control compost for 6 weeks at 7, 11, 15 or 20°C and in uninfested control compost at 20ºC. The plants were then grown outdoors in large pots at ADAS Boxworth. There were no significant differences between temperatures in verticillium incidence (mean 55% plants affected) or severity (mean index 2.25). Some verticillium developed in the uninoculated controls suggesting that there may have been some seed-borne transmission or spread between plants during the growing season.

Phoma canker was more severe at 20ºC (Index 2.84) than at 15ºC (Index 2.05), 11ºC (Index 2.14) and 7ºC (1.72).

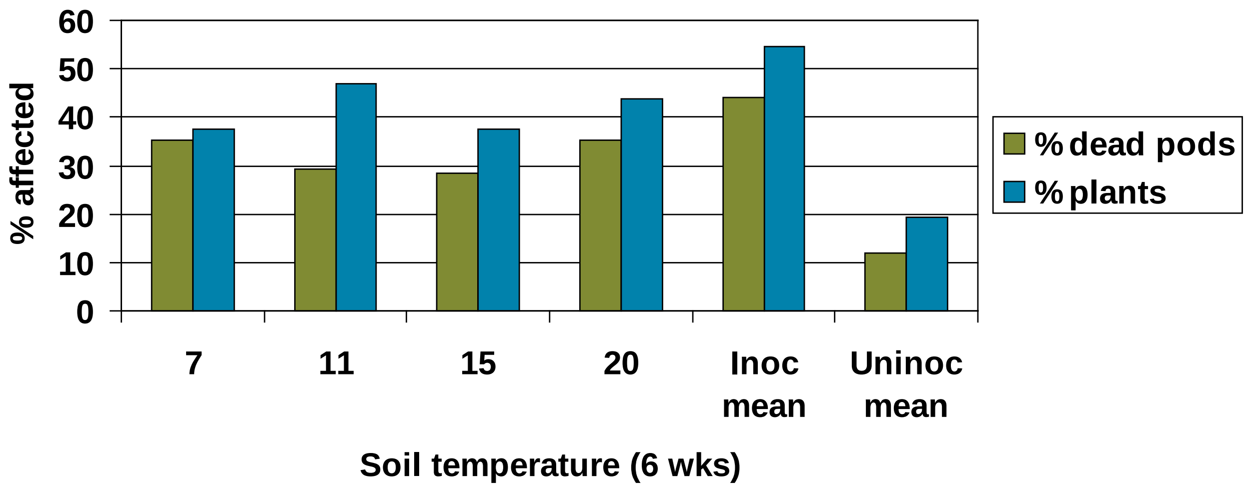


**Figure 3. Effect of different soil temperatures on the incidence (% plants) and severity (% dead pods) of verticillium wilt grown in infested compost and for inoculated versus non-inoculated compost. Young plants were grown at the 4 different temperatures (7ºC, 11ºC, 5ºC and 20ºC) in infested compost and at 20ºC in uninfested compost for 6 weeks and then grown to maturity in pots outdoors. There were no significant differences between temperatures, but inoculated compost gave more verticilium wilt (P=0.003).**

*Date of sowing pot experiment*

In the date of sowing pot experiment, there were no significant differences in verticillium incidence (mean 44% plants affected) between sowing dates (Fig. 4). Differences in severity (%dead pods) were almost significant (P=0.051, LSD would be 25.6). However, inoculation gave significantly more plants affected (43.7% compared with 18.7% in the uninoculated control; severity 26.1% dead pods compared with 6.8% in the uninoculated control). There was some verticillium developed in the uninoculated controls suggesting that there have been seed-borne transmission or spread between plants during the growing season.

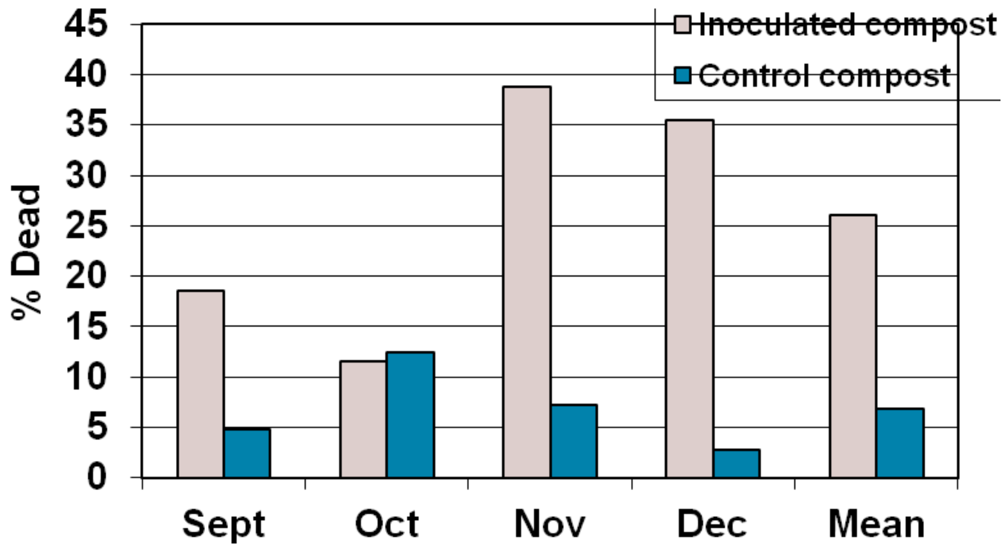


**Figure 4. Effect of date of sowing on the severity of verticillium wilt (% dead pods) in winter oilseed rape grown in pots of compost with and without inoculation with *V. longisporum.* Sowing dates were 9 September, 5 October, 3 November and 7 December. The December sowing was done in a cool glasshouse as outdoor pots were frozen. Pots were moved outdoors when seedlings had emerged and there was no risk of severe frost. There was a significant effect of inoculation but not for date of sowing.**

*Crop cover experiment*

At ADAS Boxworth, the crop sown on 3 September 2011 had started to emerge by 9 September. Subsequent growth was slow and plants remained small throughout the winter. There was no rain after 4 April and only 1.6 mm for the whole of April. This followed a dry March with only 4.8 mm of rain. In May there were 11 days with rain and 21 mm of rain in total, most of which fell after the covers had been removed on 23 May. There was 1.2 mm of rain on 6 May, 2.2 mm on 7 May with other showers giving less than 1 mm rain per day, until there was heavier rain on 26 May (6 mm) and 30 May (7.8 mm). Crop covers did not give the anticipated differential effects because of the dry spring so one of the treatments was changed to an application of 10 mm of water at the end of flowering. There were no significant treatment effects on verticillium wilt incidence (mean 60% of plants affected) or severity (expressed as % dead pods) from the cover treatments or azoxystrobin. However, there was a significant yield response of 0.35 t/ha (untreated yield 3.07 t/ha) from the azoxystrobin soil treatment (Table 11). There were no treatment differences for phoma canker or light leaf spot.

**Table 11. Yields in relation to crop cover treatments and azoxystrobin soil treatment 2010/11.**

| Trt | Actual crop cover treatment | Yield t/ha | % dead pods | % plants with verticillium |
|---|---|---|---|---|
| 1 | Uncovered | 3.13 | 15.6 | 55.0 |
| 2 | Covered at flowering | 3.04 | 23.0 | 59.0 |
| 3 | Covered at flowering | 3.34 | 22.7 | 66.0 |
| 4 | Uncovered with 10 mm water applied 25 May | 3.48 | 15.3 | 59.5 |
| | FPr | 0.276 | 0.964 | 0.479 |
| | SED (21 df) | 0.240 | 0.388 | 6.96 |
| | LSD (P=0.05) | 0.494 | 0.793 | 14.47 |
| | Soil treatment | | | |
| | Nil | 3.07 | 19.1 | 59.0 |
| | Amistar | 3.42 | 19.2 | 60.8 |
| | FPr | 0.037 | 0.955 | 0.726 |
| | SED (21 df) | 0.162 | 3.1 | 4.92 |
| | LSD (P=0.05) | 0.333 | 6.37 | 10.23 |
| | CV (%) | 14.8 | | |

*Date of sowing and variety experiments on verticillium wilt under field conditions 2011*

In Herefordshire, a time of sowing experiment was done using cvs Castille (susceptible), Catana and Ovation (both considered to have resistance to verticillium wilt). The dates of sowing were 3, 20 and 30 September 2010 and these gave significantly different mean yields of 5.06, 4.83 and 4.54 t/ha (respectively, LSD = 0.18) with no significant differences between the varieties (Table 12). Castille had a highest thousand seed weight than the other varieties and cv. Catana had larger seeds than cv. Ovation (Table 13). Thousand seed weight was significantly lower at the latest sowing. No verticillium wilt developed in the trial. Phoma canker incidence was greatest on cv. Catana and at the 20 September sowing date, although differences between all three varieties and all three dates of sowing were significant (Table 14). The differences were similar for phoma canker severity (Table 15) though there was no significant difference between the 20 and 30 September sowing dates. Phoma stem lesion incidence was significantly greater for cv. Catana on the 20 September sowing than any of the other sowing dates or cultivars. Incidence was higher for the other varieties however at the earlier sowing date. Stem lesion incidence was low across all cultivars at the later sowing date (Table 16). A similar picture is seen with phoma stem lesion severity (Table 17), with cv. Catana having significantly greater severity, and the 20 September sowing date also having significantly greater severity. Plants established well by 1 October from the first two sowings (Table 18), but the late sown treatments had a significantly lower plant population than the earlier sowings (Table 19). This experiment provided baseline yield data on the cost of delayed sowing that can be set against any yield benefit which may be found if delayed sowing decreases the impact of verticillium wilt on yield.

**Table 12. Yield of oilseed rape varieties in relation to date of sowing, Hereford 2011.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 3 September 2010 | 5.08 | 5.28 | 4.83 | 5.06 |
| 20 September 2010 | 4.68 | 4.95 | 4.85 | 4.83 |
| 30 September 2010 | 4.56 | 4.86 | 4.19 | 4.54 |
| Variety mean | 4.8 | 5.0 | 4.6 | |
| FPr | 0.097 (ns) | | | 0.029 |
| SED (24 df) | 0.130 | | | 0.130 |
| LSD (P=0.05) | 0.184 | | | 0.184 |
| Sowing date.variety FPr | 0.698 (ns) | | | |
| Sowing date.variety SED (24 df) | 0.318 | | | |
| Sowing date.variety LSD (P=0.05) | 0.657 | | | |

**Table 13. Thousand seed weight (g) of oilseed rape varieties in relation to date of sowing, Hereford 2011.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 3 September 2010 | 8.35 | 6.40 | 5.66 | 6.80 |
| 20 September 2010 | 7.95 | 5.96 | 5.73 | 6.55 |
| 30 September 2010 | 7.13 | 5.60 | 4.30 | 5.68 |
| Variety mean | 7.81 | 5.98 | 5.23 | |
| FPr | <0.001 | | | <0.001 |
| SED (24 df) | 0.179 | | | 0.310 |
| LSD (P=0.05) | 0.369 | | | 0.640 |
| Sowing date.variety FPr | 0.210 (ns) | | | |
| Sowing date.variety SED (24 df) | 0.179 | | | |
| Sowing date.variety LSD (P=0.05) | 0.369 | | | |

**Table 14. Phoma canker incidence (% plants) in oilseed rape varieties in relation to date of sowing, Hereford 2011.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 3 September 2010 | 29 | 53 | 37 | 39.7 |
| 20 September 2010 | 69 | 84 | 50 | 67.7 |
| 30 September 2010 | 65 | 67 | 40 | 57.3 |
| Variety mean | 54.3 | 68.0 | 42.3 | |
| FPr | <0.001 | | | <0.001 |
| SED (24 df) | 3.72 | | | 3.72 |
| LSD (P=0.05) | 7.68 | | | 7.68 |
| Sowing date.variety FPr | 0.011 | | | |
| Sowing date.variety SED (24 df) | 6.44 | | | |
| Sowing date.variety LSD (P=0.05) | 13.30 | | | |

**Table 15. Phoma canker severity index (0-100) of oilseed rape varieties in relation to date of sowing, Hereford 2011.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 3 September 2010 | 7.8 | 19.8 | 12.8 | 13.4 |
| 20 September 2010 | 24.8 | 39.5 | 18.0 | 27.4 |
| 30 September 2010 | 27.5 | 32.8 | 12.3 | 24.2 |
| Variety mean | 20.0 | 30.7 | 14.3 | |
| FPr | <0.001 | | | <0.001 |
| SED (24 df) | 2.34 | | | 2.34 |
| LSD (P=0.05) | 4.82 | | | 4.82 |
| Sowing date.variety FPr | 0.014 | | | |
| Sowing date.variety SED (24 df) | 4.04 | | | |
| Sowing date.variety LSD (P=0.05) | 8.35 | | | |

**Table 16. Phoma stem lesion incidence (% plants) in oilseed rape varieties in relation to date of sowing, Hereford 2011.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 3 September 2010 | 26 | 32 | 13 | 23.7 |
| 20 September 2010 | 7 | 47 | 3 | 19.0 |
| 30 September 2010 | 4 | 4 | 5 | 4.3 |
| Variety mean | 12.3 | 27.7 | 7.0 | |
| FPr | <0.001 | | | 0.002 |
| SED (24 df) | 3.54 | | | 3.54 |
| LSD (P=0.05) | 7.30 | | | 7.30 |
| Sowing date.variety FPr | <0.001 | | | |
| Sowing date.variety SED (24 df) | 6.13 | | | |
| Sowing date.variety LSD (P=0.05) | 12.65 | | | |

**Table 17. Phoma stem lesion severity index (0-100) of oilseed rape varieties in relation to date of sowing, Hereford 2012.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 3 September 2010 | 6.8 | 8.8 | 3.3 | 6.3 |
| 20 September 2010 | 1.8 | 14.8 | 0.8 | 5.8 |
| 30 September 2010 | 1.0 | 1.0 | 1.5 | 1.2 |
| Variety mean | 3.2 | 8.2 | 1.8 | |
| FPr | <0.001 | | | <0.001 |
| SED (24 df) | 1.09 | | | 1.09 |
| LSD (P=0.05) | 2.26 | | | 2.26 |
| Sowing date.variety FPr | <0.001 | | | |
| Sowing date.variety SED (24 df) | 1.89 | | | |
| Sowing date.variety LSD (P=0.05) | 3.91 | | | |

**Table 18. Plant counts at early emergence (plants/m$^2$) at Hereford on 1 October 2010.**

| Sowing date | Catana | Castille | Ovation | Sowing date mean |
|---|---|---|---|---|
| 3 September 2010 | 78.8 | 72.5 | 83.8 | 78.4 |
| 20 September 2010 | 80.0 | 55.0 | 53.8 | 62.9 |
| 30 September 2010 | 0.0 | 0.0 | 0.0 | 0.0 |
| Variety mean | 52.9 | 42.5 | 45.9 | |
| FPr | 0.12 | | | <0.001 |
| SED (24 df) | 4.94 | | | |
| LSD (P=0.05) | 0.09 | | | |
| Sowing date.variety FPr | 0.70 | | | |
| Sowing date.variety SED (24 df) | 8.55 | | | |
| Sowing date.variety LSD (P=0.05) | 17.66 | | | |

**Table 19. Plant counts (plants/m$^2$) at Hereford on 26 October 2010.**

| Sowing date | Catana | Castille | Ovation | Sowing date mean |
|---|---|---|---|---|
| 3 September 2010 | 76.2 | 67.5 | 83.8 | 75.8 |
| 20 September 2010 | 70.0 | 63.8 | 61.2 | 65.0 |
| 30 September 2010 | 45.0 | 35.0 | 43.8 | 41.3 |
| Variety mean | 63.7 | 55.4 | 62.9 | |
| FPr | 0.28 | | | <0.001 |
| SED (24 df) | 5.63 | | | |
| LSD (P=0.05) | 11.62 | | | |
| Sowing date.variety FPr | 0.70 | | | |
| Sowing date.variety SED (24 df) | 9.75 | | | |
| Sowing date.variety LSD (P=0.05) | 20.12 | | | |

*2012*

Yields in 2012 were low as the crop was severely lodged and much of the crop was flat at harvest (Tables 29 and 30). There were significant decreases in yield in the two later sowings and cv. Catana gave a significantly lower yield than cv. Castille, and cv. Castille yielded less than cv. Ovation (Table 20). The thousand seed weight of cv. Castille was significantly greater than the other two varieties (Table 21) but seed weight was not affected by date of sowing.

**Table 20. Yield (t/ha) of oilseed rape varieties in relation to date of sowing, Hereford 2012.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 2.43 | 1.88 | 3.08 | 2.46 |
| 14 September 2011 | 2.42 | 1.69 | 2.19 | 2.10 |
| 28 September 2011 | 1.98 | 1.24 | 2.54 | 1.92 |
| Variety Mean | 2.28 | 1.60 | 2.60 | |
| Fpr | <0.001 | | | <0.001 |
| SED (24df) | 0.11 | | | 0.11 |
| LSD (P=0.05) | 0.23 | | | 0.23 |
| Sowing date.variety FPr | 0.02 | | | |
| Sowing date.variety SED (24df) | 0.19 | | | |
| Sowing date.variety LSD (P=0.05) | 0.40 | | | |

**Table 21. Thousand seed weight (g) of oilseed rape varieties in relation to date of sowing, Hereford 2012.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 5.31 | 4.53 | 4.31 | 4.72 |
| 14 September 2011 | 5.25 | 4.54 | 4.23 | 4.67 |
| 28 September 2011 | 5.01 | 4.67 | 4.58 | 4.75 |
| Variety Mean | 5.19 | 4.58 | 4.37 | |
| Fpr | 0.01 | | | 0.96 |
| SED (24df) | 0.27 | | | 0.27 |
| LSD (P=0.05) | 0.55 | | | 0.55 |
| Sowing date.variety FPr | 0.89 | | | |
| Sowing date.variety SED (24df) | 0.46 | | | |
| Sowing date.variety LSD (P=0.05) | 0.95 | | | |

Verticillium wilt was found at low levels in the experiment at the pre-harvest assessment. Castile was significantly more severely affected than cvs Catana and Ovation, but was not affected by date of sowing (Tables 22 & 23). Phoma canker was present at high levels and its incidence was greatest in the late sown treatments (Table 24). Canker severity was moderate to high (indices 41-62) and is likely to have had an effect on yield. Cankers were most severe in the late sown plots (Table 25). There were no significant differences in canker incidence or severity between the varieties. Sclerotinia stem rot affected more than 30% of plants in the experiment and affected plants were dead at harvest. There were no significant treatment effects on stem rot (Table 26).

Seedling establishment was slow initially in autumn 2011 and the early sown plots only had 10 plants/$m^2$ by 3 October (Table 27). Thereafter, seedlings emerged rapidly and plant populations did not change after the assessments on 19 October when earlier sowings had at least 24 plants/$m^2$ and the late sowing averaged 45 plants/$m^2$ (Table 28).

**Table 22. Verticillium disease incidence (% plants) of oilseed rape varieties in relation to date of sowing, Hereford 2012.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 14 | 1 | 5 | 6.7 |
| 14 September 2011 | 10 | 3 | 5 | 6.0 |
| 28 September 2011 | 11 | 4 | 5 | 6.7 |
| Variety Mean | 11.7 | 2.7 | 5.0 | |
| Fpr | 0.01 | | | 0.96 |
| SED (24df) | 2.70 | | | |
| LSD (P=0.05) | 5.57 | | | |
| Sowing date.variety FPr | 0.89 | | | |
| Sowing date.variety SED (24df) | 4.67 | | | |
| Sowing date.variety LSD (P=0.05) | 9.64 | | | |

**Table 23. Verticillium wilt severity index (0-100) in oilseed rape varieties in relation to date of sowing, Hereford 2012.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 7.0 | 0.2 | 3.0 | 3.4 |
| 14 September 2011 | 3.8 | 1.0 | 1.6 | 2.1 |
| 28 September 2011 | 5.2 | 1.4 | 3.2 | 3.3 |
| Variety Mean | 5.3 | 0.9 | 2.6 | |
| Fpr | 0.02 | | | 0.647 |
| SED (24df) | 1.48 | | | |
| LSD (P=0.05) | 3.05 | | | |
| Sowing date.variety FPr | 0.85 | | | |
| Sowing date.variety SED (24df) | 2.56 | | | |
| Sowing date.variety LSD (P=0.05) | 5.28 | | | |

**Table 24. Phoma canker incidence (% plants) in oilseed rape varieties in relation to date of sowing, Hereford 2012.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 67 | 60 | 65 | 64.0 |
| 14 September 2011 | 71 | 70 | 62 | 67.7 |
| 28 September 2011 | 81 | 76 | 85 | 80.7 |
| Variety Mean | 73.0 | 68.7 | 70.7 | |
| Fpr | 0.77 | | | 0.026 |
| SED (24df) | 6.01 | | | |
| LSD (P=0.05) | 3.05 | | | |
| Sowing date.variety FPr | 0.81 | | | |
| Sowing date.variety SED (24df) | 12.40 | | | |
| Sowing date.variety LSD (P=0.05) | 21.47 | | | |

**Table 25. Phoma canker severity index (0-100) of oilseed rape varieties in relation to date of sowing, Hereford 2012.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 47.2 | 45.5 | 40.8 | 44.5 |
| 14 September 2011 | 46.5 | 42.0 | 41.0 | 43.2 |
| 28 September 2011 | 57.0 | 48.8 | 62.0 | 55.9 |
| Variety Mean | 50.2 | 45.4 | 47.9 | |
| Fpr | | 0.63 | | 0.034 |
| SED (24df) | | 5.01 | | |
| LSD (P=0.05) | | 10.34 | | |
| Sowing date.variety FPr | | 0.65 | | |
| Sowing date.variety SED (24df) | | 8.67 | | |
| Sowing date.variety LSD (P=0.05) | | 17.90 | | |

**Table 26. Sclerotinia stem rot Incidence and severity (identical values) of oilseed rape varieties in relation to date of sowing, Hereford 2012.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 39 | 36 | 36 | 37.0 |
| 14 September 2011 | 31 | 32 | 32 | 31.7 |
| 28 September 2011 | 32 | 33 | 29 | 31.3 |
| Variety Mean | 34.0 | 33.7 | 32.3 | |
| Fpr | | 0.82 | | 0.098 |
| SED (24df) | | 2.81 | | |
| LSD (P=0.05) | | 5.80 | | |
| Sowing date.variety FPr | | 0.92 | | |
| Sowing date.variety SED | | 4.86 | | |
| Sowing date.variety LSD (P=0.05) | | 10.04 | | |

**Table 27. Plant counts at early emergence (plants/m$^2$) at Hereford on 3 October 2011.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 10.5 | 17.5 | 15.5 | 14.5 |
| 14 September 2011 | 8.5 | 14 | 18.5 | 13.7 |
| 28 September 2011 | 0.0 | 0.0 | 0.0 | 0.0 |
| Variety Mean | 6.3 | 10.5 | 11.3 | |
| Fpr | | 0.05 | | <0.001 |
| SED (24df) | | 2.03 | | |
| LSD (P=0.05) | | 4.19 | | |
| Sowing date.variety FPr | | 0.28 | | |
| Sowing date.variety SED (24df) | | 3.51 | | |
| Sowing date.variety LSD (P=0.05) | | 7.25 | | |

**Table 28. Plant counts (plants/m$^2$) at Hereford on 19 October 2011.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 23.5 | 23.5 | 26.0 | 24.3 |
| 14 September 2011 | 24.0 | 29.5 | 27.5 | 27.0 |
| 28 September 2011 | 41.0 | 47.5 | 46.0 | 44.8 |
| Variety Mean | 29.5 | 33.5 | 33.2 | |
| Fpr | | 0.09 | | <0.001 |
| SED | | 1.83 | | |
| LSD (P=0.05) | | 3.79 | | |
| Sowing date.variety FPr | | 0.67 | | |
| Sowing date.variety SED | | 3.18 | | |
| Sowing date.variety LSD (P=0.05) | | 6.56 | | |

**Table 29. Lodging (% crop area affected) at harvest, Hereford 2012.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 40.0 | 40.0 | 33.8 | 37.9 |
| 14 September 2011 | 60.9 | 33.8 | 35.0 | 43.2 |
| 28 September 2011 | 37.1 | 49.0 | 56.2 | 47.4 |
| Variety Mean | 46.0 | 40.9 | 41.7 | |
| Fpr | | 0.82 | | 0.561 |
| SED (24df) | | 8.71 | | |
| LSD (P=0.05) | | 18.38 | | |
| Sowing date.variety FPr | | 0.28 | | |
| Sowing date.variety SED (24df) | | 15.09 | | |
| Sowing date.variety LSD (P=0.05) | | 31.83 | | |

**Table 30. Severely lodged crop (% crop area flat) at harvest, Hereford 2012.**

| Sowing date | Castille | Catana | Ovation | Sowing date mean |
|---|---|---|---|---|
| 2 September 2011 | 40.5 | 50.0 | 51.4 | 47.3 |
| 14 September 2011 | 47.5 | 70.0 | 50.0 | 55.8 |
| 28 September 2011 | 57.5 | 76.2 | 23.9 | 52.5 |
| Variety Mean | 48.5 | 65.4 | 41.8 | |
| Fpr | | 0.14 | | 0.763 |
| SED (24df) | | 11.62 | | |
| LSD (P=0.05) | | 24.31 | | |
| Sowing date.variety FPr | | 0.39 | | |
| Sowing date.variety SED (24df) | | 20.12 | | |
| Sowing date.variety LSD (P=0.05) | | 42.11 | | |

*Determination of yield loss from verticillium wilt*

The mean data for each of the crops used in the yield loss study show the variation in stem size, verticillium severity (expressed as percentage of pods that had ripened prematurely, termed 'dead pods') and the yield components, pod number, seed number, seed weight. There was a large variation in seed number, pod number and thousand seed weight between crops (Table 31). The Boxworth 2012 crop had a low seed count per plant, but a high thousand seed weight.

Analysis of data averaged over the five crops in relation to the verticillium index (0-5 scale) showed that there were significant differences in seed weight, seed number, seeds per pod and thousand seed weight between some of the individual disease indices (Table 32). This was mainly in comparisons of index 2 and more severely diseased plants. There was a strong correlation between the disease index and the severity of premature ripening (dead pods). This correlation was similar at all of the sites (Fig. 5).

**Table 31. Summary of yield components for each site. At each site 50 plants were assessed.**

| | Site/harvest year | | | | |
|---|---|---|---|---|---|
| Data | Boxworth 2010 | Haconby 2010 | Haconby 2012 | Boxworth 2012 | Bedford 2008 |
| Variety | Castille | Castille | DK Cabernet | Castille | Castille |
| Stem diameter (mm) | 17.3 | 13.6 | 15.4 | 13.0 | 14.5 |
| No. of pods | 224.6 | 203.6 | 385.9 | 218.5 | 246.1 |
| Total seed wt.(g) | 16.6 | 16.8 | 19.6 | 8.4 | 10.6 |
| Seed count | 3443 | 3661 | 5217 | 1080 | 2568 |
| TGW (g) | 5.1 | 4.6 | 3.7 | 7.8 | 4.0 |
| Seeds/pod | 15.3 | 18.0 | 13.0 | 4.8 | 9.8 |
| Percent dead pods | 49.6 | 19.7 | 61.5 | 43.5 | 38.1 |

**Table 32. Summary of yield components and significant effects in relation to Verticillium stem disease index. Assessments were done on 50 plants sampled from five sites (Bedford 2008, Haconby 2010, Boxworth 2010, Haconby 2012 and Boxworth 2012).**

| | Verticillium index | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Data | 0 | 1 | 2 | 3 | 4 | 5 | Fpr | Approx LSD |
| Stem diameter (mm) | 14.8 | 15.0 | 15.5 | 14.6 | 15.6 | 13.8 | n.s. | 1.478 |
| Number of pods | 256.1 | 271.7 | 300.0 | 238.9 | 260.4 | 227.3 | n.s. | 64.58 |
| Total seed weight (g) | 16.4 | 16.5 | 17.9 | 12.5 | 14.1 | 9.6 | 0.001 | 4.679 |
| Number of seeds | 3697 | 3340 | 3701 | 2659 | 3259 | 2411 | 0.075 | 596.2 |
| Thousand grain weight (g) | 4.6 | 5.7 | 5.8 | 5.3 | 5.0 | 4.4 | <0.001 | 0.397 |
| Seeds/pod | 14.7 | 11.9 | 12.2 | 10.5 | 11.9 | 10.1 | 0.001 | 2.830 |
| Percent dead pods | 0.1 | 12.1 | 32.9 | 51.0 | 83.9 | 98.8 | <0.001 | 3.525 |

*Premature ripening of pods*

The dotted regressions lines shown in Figure 5 are for individual sites. The pod loss relation (y = 0.9678x) indicates that there was an increase of nearly 10% dead pods for each 10% increase in verticillium severity. The regression lines from all the sites are very similar.

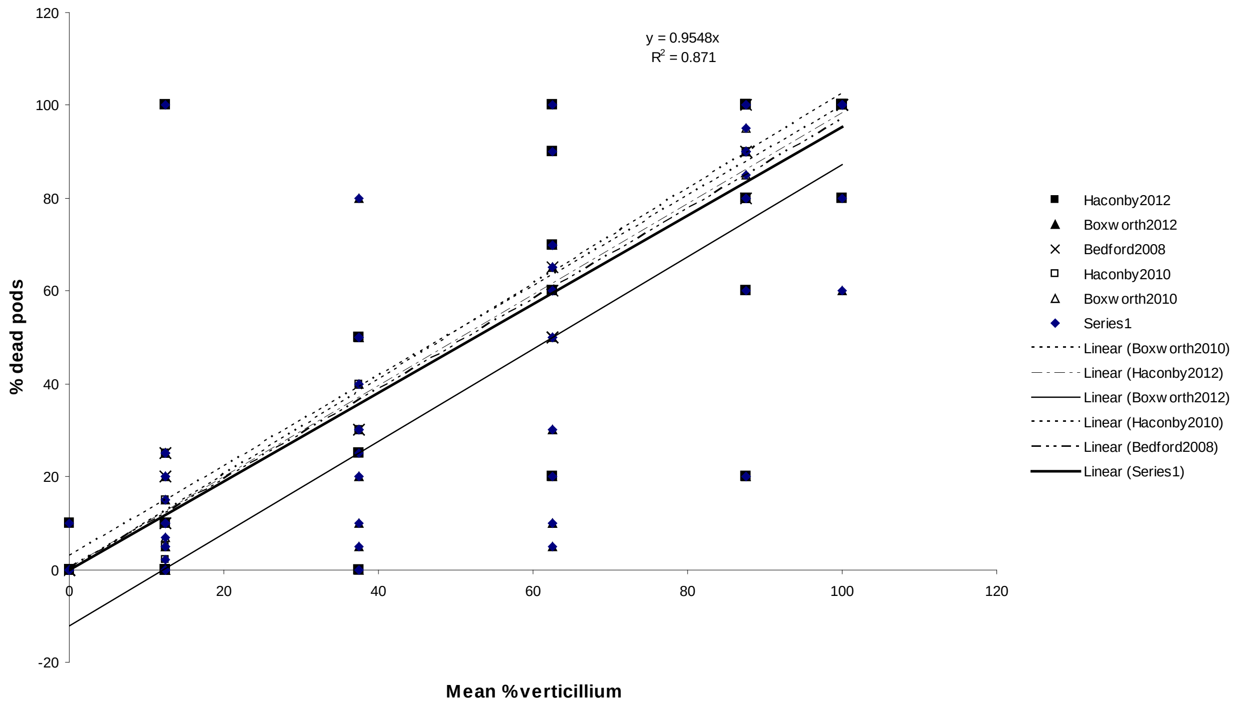


**Figure 5. Relationship between the percentage of dead pods on a single plant and the severity of verticillium wilt. Data are from five sites and regression lines have been fitted for each site. Fifty plants were assessed at each site.**

Total seed weight was related to number of pods, Fig. 6; however, this only explained 54% of the variation. Other factors including site, year and disease were also important.

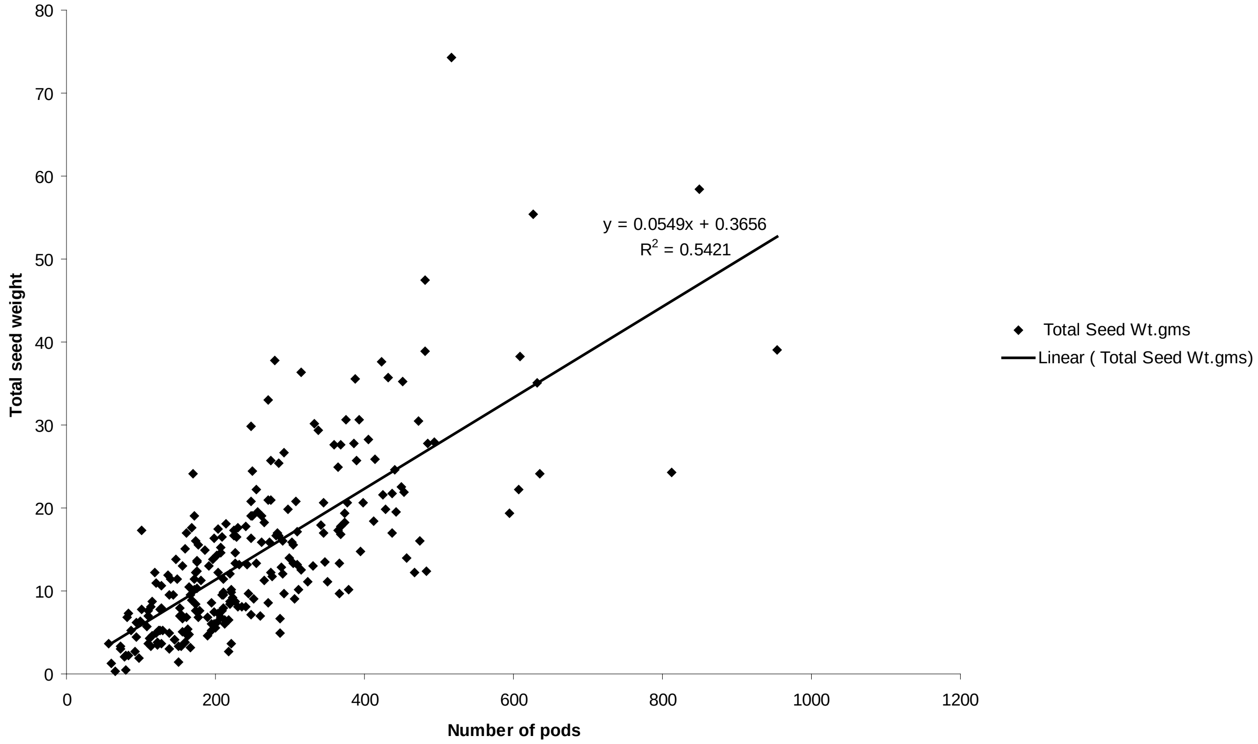


**Figure 6. Relationship between total seed weight and pod number. Assessments were done on 50 plants sampled from five sites (Bedworth 2008, Haconby 2010, Boxworth 2010, Haconbyby 2012 and Boxworth 2012).**

The main effect of verticillium was on thousand seed weight. A decrease in thousand seed weight was evident when verticillium was severe (index 4 or 5) (Fig. 7). The estimated yield loss at individual sites was in the range 12-24% (Table 33).

The crops selected were standing well and there was very little loss of seed through shedding. Losses from verticillium wilt in individual crops will depend on the severity of verticillium wilt in the stem and the extent of premature ripening. High losses have been associated with dead plants that lodged badly prior to harvest. Clearly if there is shedding of seed, losses will be greater than those estimated here which relate mainly to decreases in thousand seed weight.

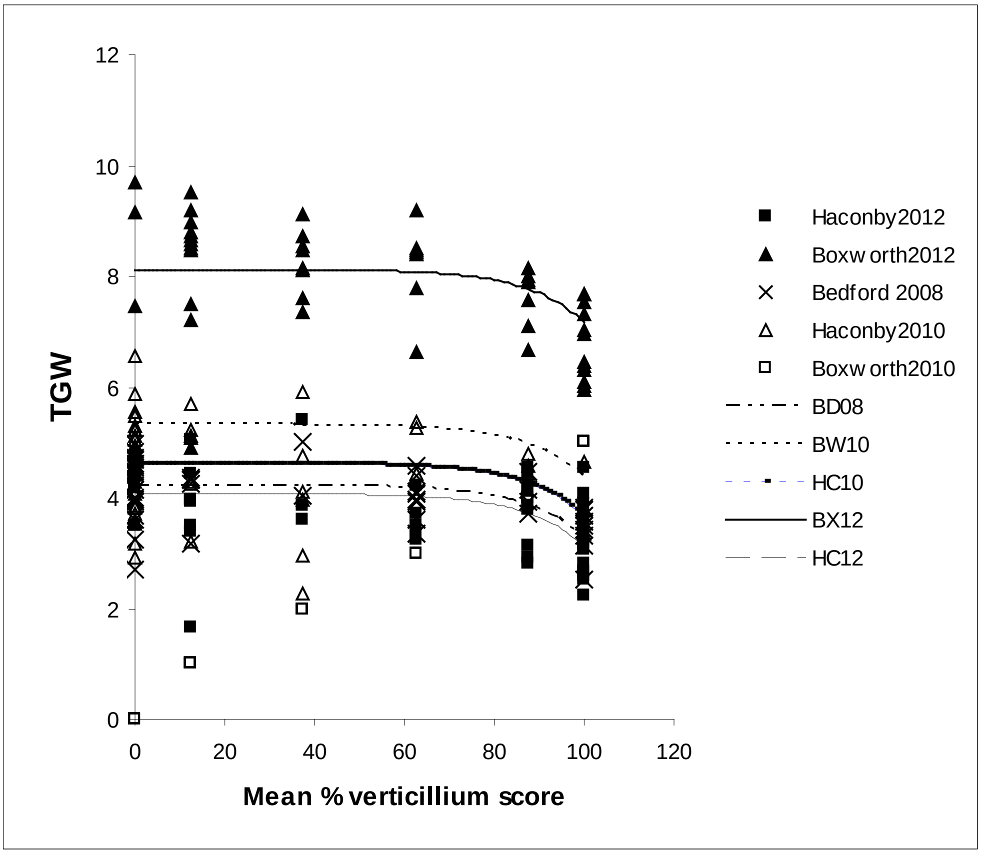


**Figure 7. Relationship between thousand seed weight (TGW) and verticillium severity score. Fifty plants were assessed at each site.**

The thousand seed weighseems to be mostly related at the top end of the distribution. Thousand seed weight loss varied between sites (Table 33).

**Table 33. Effect of verticillium wilt on thousand seed weight (TGW) of 50 plants assessed at individual sites.**

| Site/year | Boxworth 2010 | Haconby 2010 | Haconby 2012 | Boxworth 2012 | Bedford 2008 |
|---|---|---|---|---|---|
| % loss in TGW | 18.1 | 20.8 | 23.6 | 11.8 | 22.8 |

Figure 8 illustrates how yield components are related; the closely related factors have lines that are close together. Thus stem diameter is related to pod number, total seed weight is nearly inversely related to seeds/pod related to pod number. Total seed weight is made up of complex relationships between the number of seeds and the number of pods. In Figure 9, both verticillium disease measures are included and are clearly highly correlated but disease has a strong negative impact on thousand seed weight.

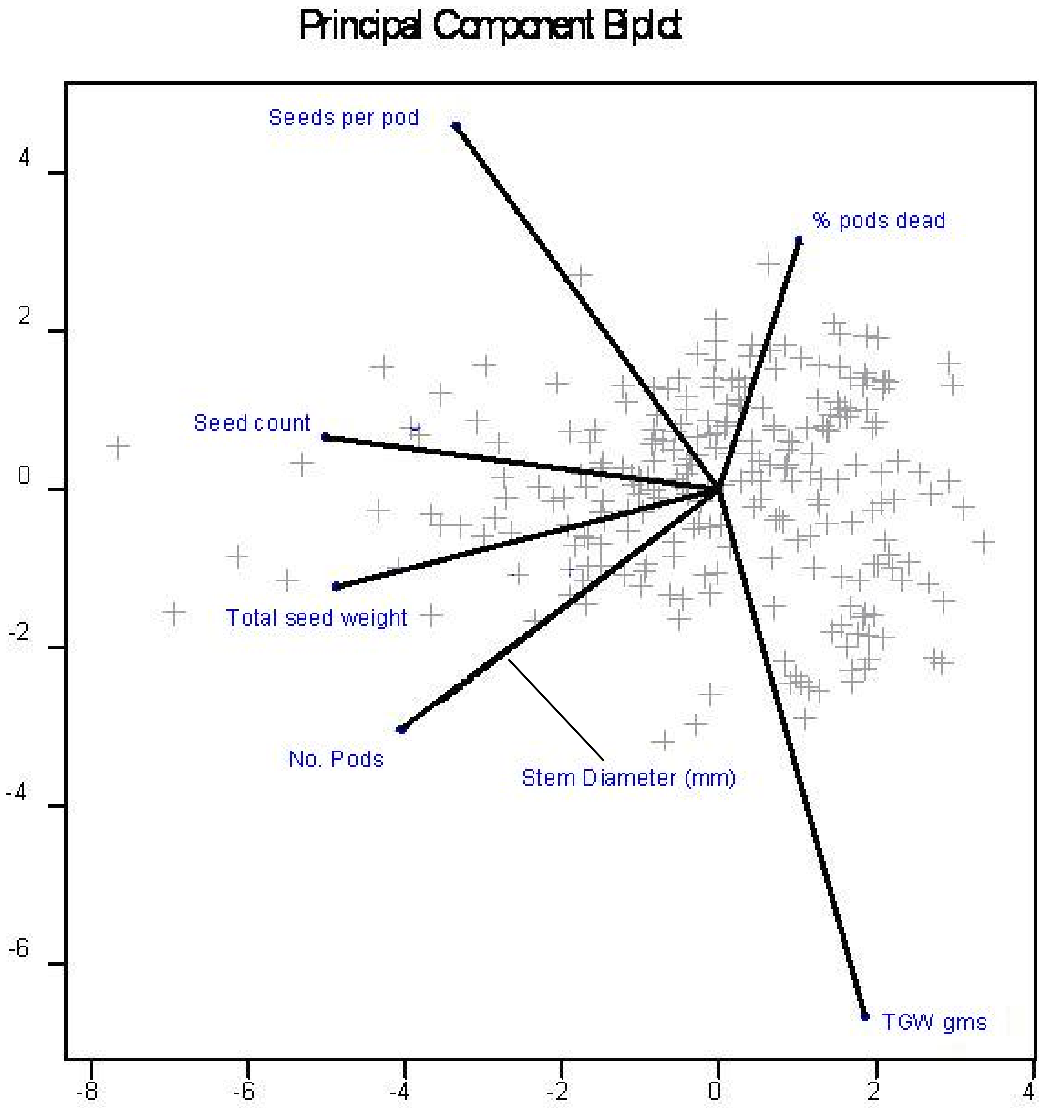


**Figure 8. Graphic representation showing relationships between yield components (seeds per pod, seed count, total seed weight, number of pods, stem diameter and total seed weight (TGW)) and verticillium wilt severity (expressed as % dead pods).**

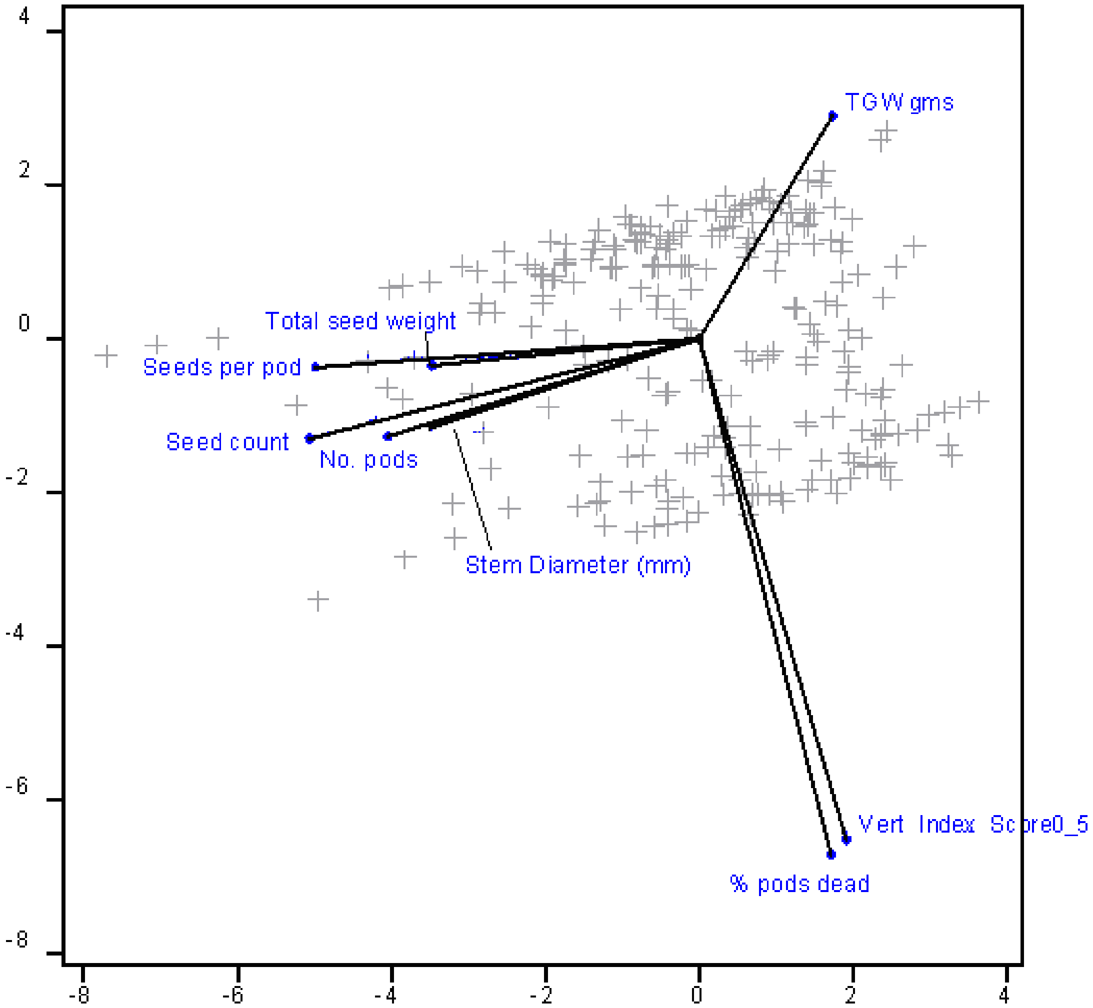


**Figure 9. Graphic representation showing relationships between yield components (seeds per pod, seed count, total seed weight, number of pods, stem diameter and total seed weight (TGW)) and verticillium wilt expressed on 0-5 index and % pods dead.**

### 3.3.3 Support plant breeding for resistance to verticillium wilt by evaluating existing varieties and novel germplasm of related crop species under glasshouse and field conditions.

*Field evaluation of winter oilseed rape varieties 2010*

The susceptibility of 24 winter oilseed rape varieties to verticillium wilt was investigated in a replicated field experiment at Bourne, Lincs. Verticillium wilt symptoms developed late in the season and first plot assessments were made just before desiccation (14 July) when seeds ranged from green-brown to brown-black (GS 6,4-6,6). There were few plants with clear external symptoms at this stage and all plants (25/plot at the ‘field end’ of the trial) were examined internally in transverse sections. There was only slight vascular discoloration in some plants and overall incidence was low at 7.8% plants affected. There were no significant differences between varieties in verticillium incidence or verticillium index, although there were differences in light leaf spot severity on stems (Table 34). Cuillin and Palace had the lowest light leaf spot scores, whilst PR45D03, PR45D06, Es Astrid and Dimension were the mostly severely affected.

A second assessment was made just prior to harvest on 26 July when external symptoms were more evident. The severity was still low and only 0.4% plants overall had severe symptoms that had killed the plant. The overall incidence was 11.9% plants affected. There were highly significant differences between varieties ($P<0.001$) for the percentage plants affected (Figure 10) and the severity index data was almost identical as nearly all plants had small lesions (data not presented). The most severely affected varieties included the widely grown cvs Castille, Excalibur and Es Astrid, and also cvs DK Secure and DK ExMen. Importantly a range of varieties had very low levels of verticillium wilt and could be considered to have some resistance to the disease. These varieties included Catana, Oracle, Alienor, Compass, Palace, Krypton and Cuillin. Many of the other varieties had intermediate levels of verticillium wilt and may have some degree of resistance. Encouragingly many of the varieties on the HGCA Recommended list (e.g. cvs Catana, Compass, Cuillin Dimension) appear to have some ‘resistance’ to verticillium wilt. However, the results should be treated with caution given this is a single experiment and further experiments are required to substantiate these observations.

The plots were assessed at each end and the separate scores plus the overall mean gave similar rankings of the varieties. The mean incidence of verticillium at each end was analysed separately and were almost identical at 11.8% and 11.9% of plants affected. For the varieties the scores were very similar (e.g. Castille 20.0% and 25.3% of plants affected, Excalibur 24.0% and 24.0% and Catana 0 and 1.3%). There was good agreement between the two sub-samples and both gave significant differences between varieties. Individual plot samples were taken to determine soil populations of verticillium so it should be possible to look at spatial variation in soil populations in due course.

Symptom expression and yield effects between varieties may be influenced by many factors including differences in maturity, prevalence of other diseases and agrochemical inputs. Concerns that symptoms in cvs Castille and Excalibur reflect their early maturity appear to be allayed by the more resistant reaction of the early maturing variety Alienor. However, data should be used cautiously before extrapolating to the wider farming environment.

Varieties were also tested in a similar experiment in another field at Bourne in the 2010/11 growing season and similar disease levels were evident pre-harvest. This experiment was not part of this HGCA project (RD-2009-3618), but disease data for the 10 varieties that were grown in both years was combined and analysed. Cvs Castille and Excalibur had significantly higher levels of verticillium wilt than the other 8 varieties (Alienor, Catana, Compass, Dimension, DK Cabernet, Flash, Palace and Sesame). This provides encouraging signs that there are consistent differences in verticillium resistance between varieties.

Scores for the first assessment were similar to the second assessment for many of the varieties, despite sampling a completely different set of plants. The second set of data also appears to be showing good consistency so a ranking of varieties can be done. The disease pressure was moderate at this site, so comparison of varietal rankings under high disease pressure is still required to find the varieties that perform consistently well in different disease environments.

There were significant differences in the incidence and severity of verticillium wilt between varieties. The established varieties Castille, Excalibur and Es Astrid were the most heavily affected; cv. Catana showed the lowest incidence. The experiment suffered seed loss in a hailstorm just before harvest and no meaningful yield data were obtained. Additional observations on varieties where disease pressure was high indicated that verticillium incidence can be very high but differences in severity (premature ripening) are still evident. Thus it is likely there are differences in resistance to verticillium wilt between varieties.  The term ‘tolerance’ has been used by some to describe varietal differences but, strictly, tolerance should refer to varieties showing lower loss of yield to a given level of disease. Tolerance to verticillium wilt may be a feature of some varieties e.g.cv. Excalibur, as farmers and agronomists report that it has generally produced good yields despite obvious symptoms of verticillium wilt. In contrast, Castille appear to suffer yield loss when severely affected. Other varieties may also suffer yield loss but this has not been investigated in this project.

**Table 34. Cultivars and verticillium and light leaf spot assessments at 14 July 2010 at Bourne, Lincolnshire. Verticilium wilt assessments were done internally in transverse sections, 25 plants per plot. A later assessment of verticilium wilt symptoms was done (see Fig. 10).**

| No | Cultivar | % plants with verticillium wilt | Wilt index (0-100) | % stem area with light leaf spot |
|---|---|---|---|---|
| 1 | Alienor | 9.3 | 1.9 | 1.17 |
| 2 | Es Astrid | 6.7 | 1.6 | 3.17 |
| 3 | Castille | 2.7 | 1.1 | 1.33 |
| 4 | Catana | 8.0 | 1.9 | 0.33 |
| 5 | Compass* | 6.7 | 1.3 | 2.27 |
| 6 | Cuillin* | 8.0 | 2.1 | 0.00 |
| 7 | Dimension* | 10.7 | 3.5 | 3.00 |
| 8 | DK Cabernet | 5.3 | 2.1 | 0.90 |
| 9 | DK ExMen* | 16.0 | 4.8 | 1.17 |
| 10 | DK Secure* | 10.7 | 4.8 | 2.07 |
| 11 | Excalibur* | 9.3 | 4.0 | 1.40 |
| 12 | Excel* | 2.7 | 0.5 | 1.77 |
| 13 | Flash* | 5.3 | 1.1 | 0.17 |
| 14 | Hammer* | 12.0 | 3.7 | 2.67 |
| 15 | Kalif | 8.0 | 1.9 | 1.07 |
| 16 | Krypton | 10.7 | 3.5 | 1.67 |
| 17 | NK Bravour | 14.7 | 4.5 | 1.00 |
| 18 | Oracle | 9.3 | 2.7 | 0.87 |
| 19 | Palace* | 2.7 | 0.8 | 0.07 |
| 20 | PR45D03* | 4.0 | 0.8 | 2.90 |
| 21 | PR45D05* | 2.7 | 0.5 | 1.67 |
| 22 | PR45D06* | 4.0 | 0.8 | 3.00 |
| 23 | PR46W21* | 5.3 | 1.3 | 1.90 |
| 24 | Sesame | 12.0 | 2.9 | 1.90 |
| | FPr | Not significant | Not significant | 0.043 |
| | SED (46df) | 2.27 | 0.11 | 1.00 |
| | LSD | 4.57 | 0.23 | 2.01 |

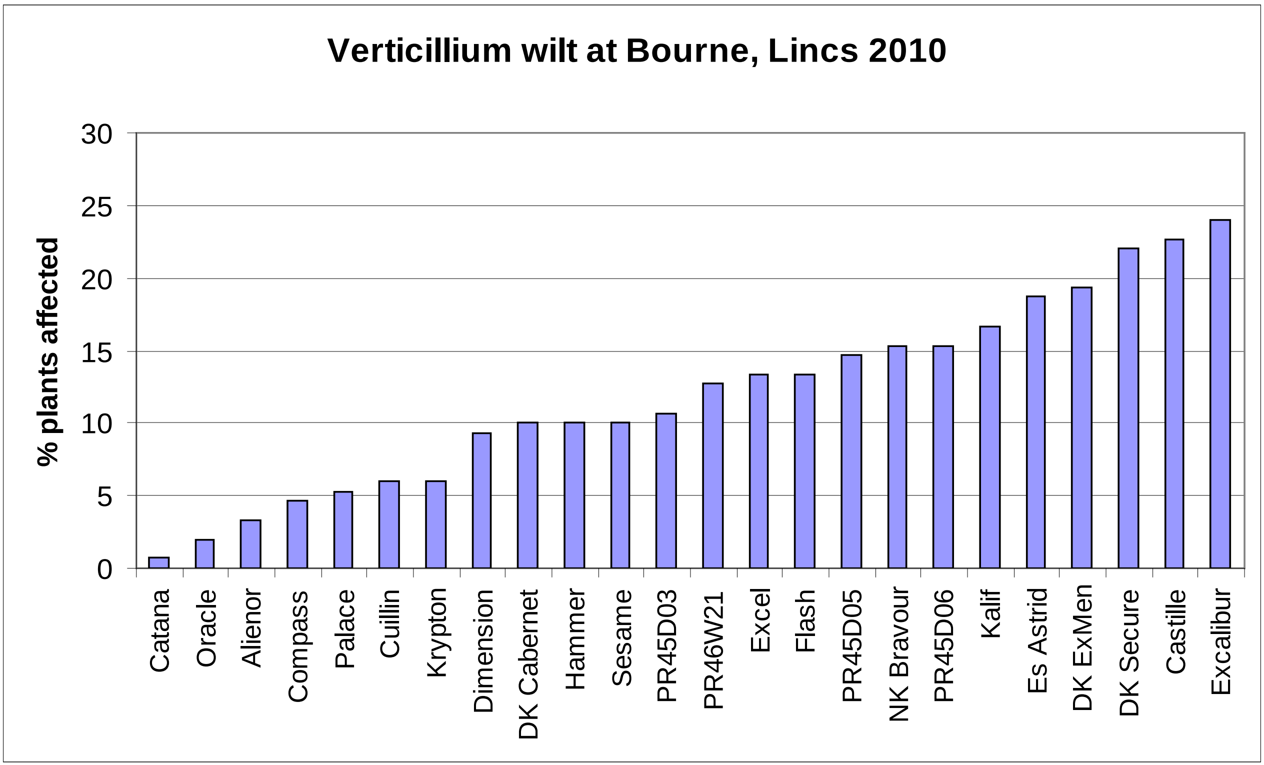


**Figure 10. Incidence of verticillium wilt in winter oilseed rape varieties 26 July 2010 (LSD = 10.2). Fifty plants per plot were assessed for internal verticilium wilt symptoms.**

The replicated variety trial at Bourne, Lincs provided some significant differences in verticillium wilt between varieties. The site has shown moderate disease levels and should be an environment where varieties can express some measure of 'resistance'. Symptoms developed late in the life of the crop and the main assessment was done on the 26 July after desiccation but whilst most stems were still green. Severity was assessed by examining a cross-section through the lower stem. It was clear that internal blackening extended well beyond the brown vertical stripes seen externally. Incidence ranged from 0.7% plants affected to 24% (LSD = 10.2). The severity index on a 0-100 scale ranged from 0.1 to 16.7 (LSD = 7.6).

A site in Kent with a range of varieties in unreplicated demonstration plots had 100% plants affected in some varieties, many of these plants were dead. The ranking of varieties grown both at Haconby and in Kent appears to be rather similar. Under the severe disease conditions there was about a 50% lower disease index score on the most 'resistant' varieties compared with the most susceptible. The incidence ranged from 66 to 100% plants affected and the severity index from 38 to 94. It is therefore likely that all varieties will become infected under high disease pressure but the more 'resistant' varieties will have less severe symptoms and show lower yield losses.

### 3.3.4 Validate molecular diagnostic tests for confirmation of plant infection and for quantification of soil-borne inoculum.

Using the standard soil extraction protocol based on *V. dahliae* and the primers that could detect both *V. dahliae* and *V. longisporum,* sensitivity of detection was approx..10-30 *V. longisporum* microsclerotia per gram in spiked soils. This was 10 fold higher than previously found for *V. dahliae* in strawberry (Bilodeau, Koike, Uribe and Martin, 2012**)**.. This is not sensitive enough for detection of *V. longisporum* in naturally infested soils where 1-3 microsclerotia per gram can cause disease in oilseed rape (Johansson, Goud and Dixelius 2006). A number of modifications of the DNA extraction method originally developed were tested, as well as published methods, but all failed to deliver increased sensitivity. As indicated above, this work was based on PCR primers which do not differentiate between *V. dahliae* and *V. longisporum*. Further work is required to determine whether this low sensitivity is due to the primers being inappropriate or the *V. longisporum* microsclerotia being more refractory to DNA extraction. In contrast, good sensitivity (down to approx. 1 microsclerotium per gram) was obtained for oilseed rape plant extracts using the same primers.

Primers designed in this project that should, theoretically, distinguish *V. longisporum* failed to detect this species in soil reliably, although at least one primer pair was specific for *V. longisporum* using DNA extracted from pure cultures. Generally, primers either lacked reliable discrimination (especially in the presence of soil extracts) or sensitivity. In the absence of quantitative soil tests for *V. longisporum*, no data were produced for thresholds or relationships between soil microsclerotial populations and yield loss.

New primers were developed which theoretically should distinguish *V. longisporum* based on previous work at Warwick Crop Centre and published papers. However, all of these lacked reliable discrimination (especially in the presence of soil extracts) or sensitivity. The primers used were based on a variety of gene sequences (both genomic and mitochondrial). We have also tried to develop primers specific for *V. dahliae* but, as expected, (*V. dahliae* being one of the parents of the hybrid isolates) it has proved very difficult to design primers with this specificity. Theoretically, primers based on mitochondrial sequences should be effective but all primers based on known mitochondrial sequences have either lacked specificity or simply not worked. In the absence of quantitative soil tests for *V. longisporum*, no data were produced for thresholds or relationships between soil microsclerotial populations and yield loss.

A total of 264 soil samples were tested for the presence of *V. longisporum* but no *V. longisporum* was detected in any of them. The fields sampled included some with a history of verticillium wilt and were therefore known to be infested. Further work is required to improve the specificity and sensitivity of test procedures.

### 3.4 Discussion

The project has contributed substantially to increased awareness and diagnosis of verticillium wilt, and has produced the first distribution map for the disease. The average level of crop infection is still quite low, although the average of 3% plant infection is greater than that of sclerotinia stem rot (*Sclerotinia sclerotiorum*) in low-epidemic years (see www.cropmonitor.co.uk). Although the map is based on almost 300 crops it does not define the distribution comprehensively, as only a few crops have been examined in some counties. Thus the survey did not record verticillium in Kent and Herefordshire, yet severe verticillium wilt has been recorded in these counties (Gladders, Smith, Kirkpatrick, Clewes, Grant, Barbara, Barnes and Lane, 2011). The map should be used as a guide to the relative distribution with areas shown as ‘0’ having some infected crops. Conversely some of the heavily infested areas in eastern England may have a greater proportion of affected crops than the current 30-40% of crops indicated by the survey. The distribution map will need to be updated in future years and this will be facilitated by the inclusion of verticillium wilt in the June survey assessments at Fera. In the 2012 survey, CropMonitor reported verticillium wilt in 10% of crops nationally and 1.4% plants affected, again mostly in the east (18% crops) with some in the southeast (8% crops) and north (9% crops). The survey samples were taken in late June and may underestimate verticillium wilt as symptoms continue to develop up to harvest. Survey samples consist of 25 plants so low levels of verticillium wilt (<4%) would not be detected reliably.

Agronomists and farmers should inspect crops close to harvest for signs of verticillium wilt and quantify its prevalence in each field. Future decisions on oilseed rape cropping, varieties, agronomy and rotation can then be adjusted to reduce the risk of yield loss from verticillium wilt. There is little survey information from other countries in Europe so it has not been possible to make comparisons of disease distribution or rates of spread.

There are likely to be various factors that influenced the distribution of verticillium wilt, particularly the concentrated distribution in parts of eastern England. This heavily infested area was one of the first to adopt winter oilseed rape cropping in the 1970s. The soil type is predominantly clay loam with a predominance of wheat and oilseed rape cropping with shorter winter oilseed rotations. The survey data indicated that verticillium wilt is present in winter oilseed rape in a range of rotations so as yet, short rotations are not proven to be most severely affected. However, the single cases of oilseed rape being grown as a second successive crop in 2009 and 2011 highlighted this as a high risk situation as in the 2011 crop 73% of plants had verticillium. There may well be climatic factors involved, with dry autumns giving poor or late establishment (at least until the advent of subsoil drilling techniques) and dry summers putting plants under drought stress. As some fields are heavily and uniformly infected, it is likely that the pathogen has been present for several years. There is potential for *V. longisporum* to be introduced on seed as internal seed-borne infection or as a contaminating dust on the outside of seed. External seed contamination was confirmed by during the project, on seed harvested from a heavily infected crop (D Barbara pers. comm.).. This raises concerns that home-saved seed from infected crops could be contributing to the spread of verticillium wilt. A seed test for *V. longisporum* would be a useful addition to the existing range of seed test available to farmers. The effectiveness of seed treatments against *V. longisporum* is not known. Recent research suggests that seed treatments with antagonistic bacteria may be able to contribute to control of verticillium wilt (Abuamsha, Salman, Ehlers, 2011). Novel approaches as well as fungicidal treatments should be evaluated for control of verticillium as a priority.

The first variety trial produced a clear ranking of varieties for resistance to verticillium wilt. The broad pattern has been substantiated outside this project in more recent experiments and demonstration plots. The project has had a strong impact on varietal selection, notably the move away from susceptible cv. Castille on farms with verticillium wilt since autumn 2010.  Farmers and agronomists have taken interest in the more resistant varieties and increased use of cvs Catana and Alienor on some infested farms. The disease pressure at the Haconby site was low to moderate. At other sites with high verticillium pressure, the differences between varieties have still been evident with most plants of varieties such as Castille being dead whilst plants of others such as Catana had verticillium wilt but remained green up to desiccation. Even varieties with very low levels of disease, which could be thought of as being ’resistant’, are not immune to verticillium wilt and if disease pressure is high could show some premature ripening. Whilst use of resistant varieties is an obvious step in the management it may not be sustainable in very short rotations.

Breeders are now collating information from their own experiments in the UK and elsewhere in Europe. Resistance to verticillium wilt is polygenic and therefore should not be broken suddenly as can occur with other diseases e.g. rust resistance in wheat. Resistance to verticilium wilt in cauliflower appeared to restrict spread and development of the pathogen (Debode, Declercq and Höfte*.,* 2005). Sources of resistance have also been identified in cabbage and in *B. rapa* (Rygulla *et al.,* 2007). Four different chromosome regions with quantitative trait loci (QTL) have been identified, with the two major QTL regions being on the C-genome which is therefore likely to have originated from *B. oleracea* (Rygulla, Snowdon, Friedt, Happstadius, Cheung and Chen*.,* 2008). Obermeier et al., (2012) have reported that some of the QTLs are associated with phenylpropanoid metabolism which is associated with resistance. The aim is to combine several resistance loci to produce resistant genotypes. Mapping populations have been produced and molecular markers need to be identified to assist the selection of resistant loci (Rygulla *et al*., 2007, Rygulla *et al.,* 2008). A number of oilseed rape breeders are now starting to take account of verticillium wilt resistance in their breeding programmes and this will receive higher priority when yield losses occur more frequently.

The single plant studies demonstrated that plants with severe verticillium wilt (> 50% stem circumference affected) produced smaller seeds. Other yield components such as pod number and number of seeds per pod may be affected if there is symptom development during flowering but, at present, most symptoms appear towards the end of June and in July. There were several reports of verticillium causing premature ripening and loss of yield in eastern and central England in 2012. Typically losses were estimated by farmers at 10%, occasionally more. Severe losses have been reported from crops where the stems were heavily infected and weakened, so that the crop canopy collapsed. Under these conditions losses are due to seed shedding as well as decreased seed size.

The highest incidence during 2009-2011 UK surveys was in 2010. This may be due to chance but this was the year with the highest proportion of late August sown crops. The date of sowing and soil temperature experiments indicate that verticillium is able to affect plants over a long period but very early sowing dates were not tested . Further work is required to examine sowings in early to mid August as these have apparently increased verticillium severity in Germany (Kreye, Steinbach and Wolf, 2006). Extending the growing season from a standard 45 weeks (315 days) by 1-3 weeks increased substantially both disease incidence and microsclerotial populations in soil (Kreye, Steinbach and Wolf, 2006). Weather patterns during the growing season are likely to influence verticillium wilt.  Temperatures from May until harvest are thought to have influenced final disease severity In Germany (Dunker, Keunecke, Steinbach and von Tiedemann, 2008; Soesanto and Termorshuizen, 2001). The severe attacks in 2012 in eastern England occurred despite cool wet conditions from April onwards, but followed an autumn period when plants grew vigorously and above average temperatures in March. Further monitoring and experimentation is required to define combinations of temperature and rainfall that influence the severity of verticillium epidemics.

In 2011, symptoms of verticillium wilt appeared from mid-June onwards, but most developed close to harvest. A few crops showed a high incidence of severely affected plants and these crops usually had poorly developed root systems or were growing on shallow, drought prone soils. Field observations suggest that good crop agronomy can contribute to decreasing the impact of verticillium wilt as strong plants will tend to show less premature ripening than ‘stressed’ plants. Where verticillium wilt is present on farms, good crop establishment and strong rooting are likely to be beneficial for its management. Further steps can be taken by selecting varieties with good resistance to verticillium wilt before decisions need to involve longer rotations of oilseed rape (minimum of 1 year in 4).

Specific and sensitive PCR-based detection of *V. longisporum* (especially in soil) is clearly challenging. Although several different sets of primers and DNA extraction methodologies were tested, none were suitable for a reliable soil or plant test. However, in a separate project, Warwick Crop Centre has generated whole genome sequence from a UK *V. longisporum* isolate. This was compared with two other strains of *V. longisporum* sequenced by Susanna Braus-Stromeyer from the University of Gottingen in Germany as well as one *V. dahliae* and one *V. albo-atrum* whole genome sequence. This has allowed several further pairs of primers to be designed with potential specificity to *V. longisporum* mitochondrial DNA sequence. In addition, recent publications may also provide the basis for designing new *V. longisporum* specific primers (Inderbitzin, Bostock, Davis, Usami, Platt and Subbarao 2011; Inderbitzin, Davis, Bostock and Subbarao. 2011b). Work is required to evaluate the reliability, specificity and sensitivity of these potentially useful primers.

*Future priorities for research and knowledge transfer on verticillium wilt*

There is no published list of resistance ratings for verticillium wilt and the industry therefore lacks clear guidance for selecting varieties for fields with high levels of verticillium wilt. A number of experiments will be required to produce robust ratings suitable for publication as part of the Recommended List. Standard protocols for assessing vertcillium wilt in variety experiments should be developed.

Further monitoring of verticillium and updated distribution maps should be produced in conjunction with CropMonitor and Defra-funded disease surveys.

The importance of seed-borne infection should be investigated. Rapid quantitative seed test methods should be developed so that seed stocks can be tested quickly after harvest.

The development of a soil test for Verticillium wilt requires further funding. This is of fundamental importance for quantifying changes in soil populations in different rotations.

Knowledge transfer to increase awareness of verticillium wilt and how it can be managed should continue to be supported. There is still time for decisions on farms to influence the build up and impact of this disease.

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### 3.5.1 Site details for Verticillium wilt experiments ADAS Boxworth.

*Winter oilseed rape verticillium crop covers site, 2010/11.*

| | | | |
|---|---|---|---|
| Site: | Boxworth, Cambs | | |
| Field name: | 40 Acre | | |
| Soil texture: | Clay loam | | |
| Drainage: | Good | | |
| Previous cropping: | 2010 Winter wheat | | |
| | 2009 Winter wheat | | |
| | 2008 Winter beans | | |
| | 2007 Winter wheat | | |
| Soil analysis: | pH 7.4 | | |
| (November) | ADAS Indices – P 19.4 mg/l (2), K 276 mg/l (3), Mg 104 mg /l (3) | | |
| | 2.9% organic matter | | |
| Crop: W. oilseed rape | Cultivar | : | Castille |
| Sowing date | | : | 25 August 2010 |
| Seed rate | | : | 80 seeds/m$^2$ |
| Fertiliser (kg/ha) | Urea 46% | 130 | 9 march 2011 |
| | As 21:0:0:60 | 100 | 16 February 2011 |
| | Urea 46% | 215 | 24 March 2011 |
| Herbicides: | Aramo (1.0 l/ha) 12 October 2010 | | |
| | Kerb (2.1 l/ha)) on 18 November 2010 | | |
| | Galera (0.35 l/ha) on 11 March 2011 | | |
| Fungicides: | Pre Drill – Amistar (6l/ha) | | |
| | Overspray – Sanction (0.40 l/ha) | | |
| | Plover (0.3 l/ha) 25 October 2010 | | |
| | Sanction (0.4 l/ha) 8 February 2011 | | |
| Insecticides: | | | |
| Molluscicides: | 8.0 kg/ha on 17 September 2010 | | |
| Growth regulator: | | | |
| Trace elements: | | | |
| Desiccant | Glyphosate ?2.0l/ha on 4 July 2011 | | |
| Harvest date: | 25 July 2011 | | |

*Spray application details and conditions at spraying 2010/11.*

**Table 35. Application details, Boxworth 2010/2011.**

| Actual date | Products applied | Growth Stage | Weather |
|---|---|---|---|
| 3 September 2010 | Amistar | Pre-em | Sunny and cloudy, crop was dry.<br>RH 58.6-58.9%<br>Very slight drift, wind speed 1.6-1.7mph |
| 31 January 2011 | Sanction | | Dry and cool conditions.<br>RH 77.5-77.7%<br>Wind speed 0-2mph<br>No drift |

*Spray application equipment*

Sprayer: OPS with 3 m boom

Nozzles: F110.

Water volume: 200 litres

Pressure: 2.0 bar

### 3.5.2 Site details for ADAS Rosemaund winter oilseed rape verticillium time of sowing site, 2011/12.

| | | | |
|---|---|---|---|
| Site: | Bishops Frome, Hereford | | |
| Field name: | Wooton Farm | | |
| Soil texture: | | | |
| Drainage: | | | |
| Previous cropping: | | | |
| Soil analysis: | pH 6.3 | | |
| (November) | ADAS Indices – P 12.2 mg/l (2), K 98 mg/l (3), Mg 174 mg /l (3) | | |
| | 1.9% organic matter | | |
| Crop: Wr oilseed rape | Cultivar | : | Castille, Catana, Ovation |
| Sowing date | | : | 02/09/2011 |
| | | | 14/09/2011 |
| | | | 28/09/2011 |
| Seed rate | | : | 70 seeds/$m^2$ |
| Fertiliser (kg/ha) | | | |
| Herbicides: | | | |
| Fungicides: | Overspray: Filan (0.250 l/ha) | | |
| Insecticides: | | | |
| Molluscicides: | | | |
| Growth regulator: | | | |
| Trace elements: | | | |
| Desiccant | | | |
| Harvest date: | 14/08/12 | | |

*Spray application details and conditions at spraying 2011/12.*

**Table 36. Application details, Rosemaund 2011/2012.**

| Actual date | Products applied | Growth stage | Weather |
|---|---|---|---|
| 24/04/12 | Filan | Early-mid flower | Dry, overcast and cool. Dry crop, 100% ground cover. 12.8 – 13.2$^{o}$C, RH 76.4 – 82.6% Wind speed 2.1 – 2.3 mph. |

*Spray application equipment*

Sprayer: OPS with 3 m boom

Nozzles: 03F110

Water volume: 200 litres

Pressure: 2.0 bar

### 3.5.3 Site details ADAS Rosemaund winter oilseed rape verticilium time of sowing site, 2010/11

| | | | |
|---|---|---|---|
| Site: | Bishops Frome, Hereford | | |
| Field name: | By Fred's | | |
| Soil texture: | | | |
| Drainage: | Good | | |
| Previous cropping: | Winter wheat | | |
| Soil analysis: | pH 6.3 | | |
| (November) | ADAS Indices – P 12.2 mg/l (2), K 98 mg/l (3), Mg 174 mg /l (3) | | |
| | 1.9% organic matter | | |
| Crop: W oilseed rape | Cultivar | Various | Castille, Catana, Ovation |
| Sowing date | | : | 03/09/2011 |
| | | | 20/09/2011 |
| | | | 30/09/2011 |
| Seed rate | | : | 100 seeds/$m^2$ |
| Fertiliser (kg/ha) | | | |
| Herbicides: | Novall (2l/ha) | | |
| Fungicides: | | | |
| Insecticides: | | | |
| Molluscicides: | | | |
| Growth regulator: | | | |
| Trace elements: | | | |
| Desiccant | | | |
| Harvest date: | 17/08/11 | | |

*Spray application details and conditions at spraying 2010/11.*

**Table 37. Application details, Boxworth 2010/2011.**

| Actual date | Products applied | Growth stage | Weather |
|---|---|---|---|
| 03/11/10 | Novall (2l/ha) | 1.0 – 1.1 | Dry and cool conditions.<br>Dry crop, 10% ground cover.<br>14.5$^{o}$C, RH 71.2 – 75.4%<br>Wind speed 5.7 mph<br>Slight drift. |

*Spray application equipment*

Sprayer: OPS with 3 m boom

Nozzles: 03F110

Water volume: 200 litres

Pressure: 2.0 bar

### 3.6.4 Site details Verticillium wilt variety trial Haconby, Lincs 2009/2010.

| | | | |
|---|---|---|---|
| Site: | Haconby, Linconshire NG34 0TL | | |
| Field name: | Mills 15 | | |
| Soil texture: | Silty loam | | pH 7.5 |
| Drainage: | Good | | OM 5.3% |
| Previous cropping: | 2009 Wheat | | |
| Soil analysis: | K 382 (mg/l), P 26 (mg/l), Mg 179 (mg/l) | | |
| | K index: 3, P Index: 3, Mg index: 4 | | |
| (November) | | | |
| Crop: Wr oilseed rape | Cultivar | : | Various – see Table 38 |
| Sowing date | 28 August 2009 | : | |
| Seed rate | See Table 38 | : | |
| Fertiliser (kg/ha) | | | |

| | |
|---|---|
| Herbicides: | Aramo (1.0 l/ha) 23/9/2009 |
| | Springbok (2.5l/ha) 30/09/2009 |
| | Kerb Flo (2.1 l/ha) 10/11/2009 |
| | Nufosate Ace (4l/ha) 12/07/2009 |
| Fungicides: | Corinth (0.75l/ha) 19/10/2009 |
| | Proline 275 (0.4 l/ha) + Prosaro* (0.8 l/ha) 17/03/2010 |
| | Amistar (0.8 l/ha) 19/04/2010 |
| | Delsene 50 Flo (0.5 l/ha) + Proline 275 (0.45 l/ha) 19/04/2010 |
| | Filan (0.2l/ha) 17/05/2010 |
| | * Farm crop only |
| Insecticides: | Karate (2.5 l/ha) on 13/10/2009 |
| Adjuvants | X-Change (0.25 l/ha) 23/9/2009, 01/03/2010, 12/07/2010 |
| | Silwet (0.1 l/ha) 10/11/2009 |
| | Pod-stick (1l/ha) 12/07/2010 |

| | |
|---|---|
| Trace elements: | Mn 15% Lenric (3l/ha) 13/10/2009 |
| | Mn 15% Lenric (4l/ha) 17/03/2010 |
| | Mn 15% Lenric (5l/ha) 17/03/2010 |
| | Nutriphite PGA (0.5l/ha) 13/10/2009 |
| | Headland Boron 15% (3l/ha) 19/10/2009 |
| | Headland Boron 15% (2.5l/ha) 01/03/2010 |
| | Phorce (1l/ha) 17/03/2010 |
| Harvest date: | 03/08/2010 |

### 3.6.5 Primers tested in the project

Table 38. List of potential *V. longisporum* specific primer sets tested in this project.

| **Primer** | **Sequence** | **Target gene region** |
|---|---|---|
| Vd-V F1 | CCGTTTCCCGTTACTCTTCT | ITS |
| Vd-V F2 | TAAAAGTACTATATATACTTGAACG | ITS |
| Vd-V | RGCAATGTATTTCGCTAGGCC | ITS |
| | | |
| VL-V F1 | GCCGTTTCTCGCTATTCTTTC | AH alpha |
| VL-V F2 | AAGTACTGTATGCACTCTACTA | AH alpha |
| VL-V | RATTGCAATACATTTCTTCTAGTG) | AH alpha |
| | | |
| VL-5.8 F1 | CCGCAAGGCCAATCGCCC | 5S rRNA IGS |
| VL-5.8 F2 | GAAAGATGTGCTCGGAATTGT | 5S rRNA IGS |
| VL-5.8 R1 | TCGAGTGCTCGTTCGCTAC | 5S rRNA IGS |
| VL-5.8 R2 | GCATCTGAGAGCCTCTTACAA | 5S rRNA IGS |
| Cyto B Vd F1 | ATTATCTGAGGAGGTTTTTCAG | Cytochrome b intron |
| | | |
| Cyto B Vd R | GCTGCTAACATAGCTATAACAC | Cytochrome b intron |
| Cyto B Vd F2 | GTGTTATAGCTATGTTAGCAGC | Cytochrome b intron |
| NADH Vd F | GAGTTTACTTATAATTCGTTCTTC | NADH dehyd sub5 intron |
| NADH Vd R1 | ATATCTCCTTCATGGCTAACAT | |
| NADH Vd R2 | GAAGAACGAATTATAAGTAAACTC | |
| | | |
| VL-INT F | GACCTCTGGTCATGACTCC | Intron from Karapapa and Typas (2001) |
| VL-INT R | ACGACATACTCGCTCCCGT | Intron from Karapapa and Typas (2001) |
| | | |
| V12E.03F | TCTCCTCTCTACGAGAACGA | OSR isolate specific genomic sequence from Steventon et al (2002) |
| V12E.03R | CACTTTCTAAGTATCCTTCCTAT | OSR isolate specific genomic sequence from Steventon et al (2002) |