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Final Project Report 605

Evaluation of resistance levels to verticillium wilt in UK oilseed rape varieties and relevance to productivity

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CONTENTS

1. Abstract

This project aimed to determine whether consistent and reproducible differences in the levels of verticillium wilt infection occur between oilseed rape varieties on the AHDB Recommended List (RL). Inoculated or naturally occurring infection tests were used to determine the relevance of infection to oilseed rape production, in terms of the yield loss incurred. Such information could be potentially be used to devise disease resistance ratings or rankings on the RL.

Over the three years of testing, highly consistent differences between varieties, in terms of the level of symptom expression, were observed. These occurred regardless of whether trials were inoculated with fungal microsclerotia or established on land associated with high levels of infection. Objective scoring methodology was devised that would be appropriate for use in variety evaluation systems.

Clear and significant differences in disease levels were observed between varieties and, though none was found to be completely resistant, several showed consistently low infection levels. This differentiation allowed the selection of three varieties with high levels of partial resistance and three more susceptible varieties for inclusion in four trials to investigate the effects of verticillium on yield. Inoculation of blocks of land was used to create differentiation in disease pressure on the resistant and susceptible varieties. Though yield effects were variable, losses were larger in the trials with clear differences in disease levels between inoculated and non-inoculated blocks. Susceptible varieties showed significantly greater yield depression in one trial, and resistant varieties showed little or no effect throughout all trials. The project has shown that variety reactions to verticillium infection are significantly and consistently different, and that the differences are great enough to enable a rating system to be introduced. Selecting resistant varieties on land with known high levels of infection is highly likely to mitigate against yield loss. Visible production of microsclerotia was also less on more resistant varieties, and this factor could also contribute towards disease management, by reducing the return of disease to the soil.

2. Introduction

Verticillium longisporum was first confirmed on winter oilseed rape in the UK in 2007. Initially termed 'Verticillium wilt', the name Verticillium stem stripe was proposed in 2016 (Depotter *et al*., 2016) and has become increasingly adopted in the scientific literature. The change in common name was prompted by the fact that the pathogen does not cause typical wilting symptoms in the field. Occasional leaf yellowing may be seen, normally affecting only one side of the leaf lamina, but significant symptoms are not seen until the end of the season, usually towards the end of June and early July. At this time, unilateral brown striping on the stems is visible. This progresses to a final phase where the stem begins to shred, with masses of minute black microsclerotia appearing in the tissue (see Figure 1). The microsclerotia become incorporated with plant debris in the soil where they can persist for over 10 years (Heale and Karapapa, 1999). Germination is stimulated by root exudates of the next oilseed rape crop, and root and stem base tissue becomes infected. Other brassicacea, including vegetable brassicas, can also be infected. The fungus finally colonises the vascular tissue and, depending on which vessels are infected, the typical unilateral striping occurs.

Figure 1. Unilateral brown stem stripes (left) and late stage stem shredding with microsclerotial development (right). Photos courtesy ADAS and NIAB respectively.

Since its first occurrence, Verticillium has been reported sporadically in oilseed rape crops, occasionally causing widespread infection within a crop. In 2011, routine monitoring began in the Defra Crop Disease summer survey (www.cropmonitor.co.uk). Incidence has varied significantly, but just over one fifth of crops nationally have been affected in three of the past eight years, with up to 40% of crops affected in some regions. Severity within crops on average has been low at up to 5% stems infected. However, samples are collected in June, which is usually too early to see full expression of Verticillium symptoms.

The occurrence, impact, and biology of *Verticillium longisporum* in Europe has been recently and extensively reviewed (Depotter *et al*., 2016). The organism is a hybrid between two unknown progenitor Verticillium species (termed A1 and D1), and has a restricted host range on brassicaceous plants. The reported effects on oilseed rape yield have been variable, but all indicate that substantial effects occur (see Table 1).

Table 1. Summary of the range of yield effects reported for oilseed rape infected by Verticillium.

Country	Method	Yield loss estimate	Reference
UK	Single plant comparisons	12-24%	Gladders *et al*., 2013
Sweden	Infected vs. non-infected plot	44%	Svennson & Lerenius, 1987
Germany	Single plant comparisons	10-50%	Duncker *et al*., 2008
UK	Infected vs. non-infected plot	34%	Depotter *et al*., 2018
UK	Infected vs. non-infected plot	0-13%	Thomas & Wood, 2014

Currently, there are no fungicides available with specific label recommendations for the control of Verticillium on oilseed rape. Widening rotations between crops may help to reduce levels of microsclerotia in soil, but since these have long-term persistence, rotational practice alone is unlikely to be an effective control measure. These circumstances have therefore driven interest in variety resistance, and whether more susceptible varieties suffer significant yield losses and conversely whether any available resistance is sufficient to mitigate against the effects of the disease. Gladders *et al*., (2013) and Thomas & Wood (2014) reported significant variation in the level of symptom expression between varieties of commercially grown winter oilseed rape in the UK. However, there is currently no system for producing data each year for varieties on the AHDB Recommended List, thus growers have little guidance on the potential for selecting more resistant types. This project was undertaken to investigate the resistance of AHDB Recommended List varieties, and to understand the impact of *V. longisporum* over sites and seasons on the productivity of more resistant compared with more susceptible varieties. Depending on experimental outcomes, the project aimed to propose systems for the production of resistance ratings for grower information.

3. Materials and methods

3.1 Establishment of resistance screening experiments

AHDB Recommended List varieties, including year-one candidates harvested in each of the project sowing years, were used in experiments established in autumn 2015, 2016, and 2017, with 44, 52 and 46 varieties respectively, and four replicates in each experiment. A set of 22 varieties was common to all years. Putative control varieties, which limited previous data (Gladders *et al*, 2013, Thomas & Wood, 2014) had indicated were partially resistant (Incentive and Catana) or more susceptible (Harper and Quartz), were included. Two varieties with putative intermediate resistance (DK Cabernet and PR46W21) were also included. Seed of Quartz was unavailable for autumn 2017 sowing. In each year, one experiment managed by ADAS was sown at a site in eastern England where there had been a very high level of Verticillium in preceding oilseed rape crops. The other experiments, managed by NIAB, were sown in land with no previous history of the pathogen, also in eastern England, and were inoculated 1–2 weeks after sowing. Inoculum consisted of a maizemeal and vermiculite substrate which had previously been infected with UK isolates of *V. longisporum* and incubated at room temperature until abundant microsclerotia had formed. The mixture was air dried before spreading on the test plots, using an amount calculated to give between 40–100 colony forming units cm^{-2} of soil. The experiments received a full crop protection programme of fungicides, herbicides and pesticides, including available insecticides for flea beetle control. One inoculated experiment was lost in autumn 2016 due to cabbage stem flea beetle, and a replacement was established at an additional, separate site in autumn 2017. Site details are summarised in Table 2.

Table 2. Site details for resistance screening experiments.

Plot size	Sowing date	Inoculation	Location	Variety number
12 m x 3 m	27 August 2015	No	Boxworth, Cambs. (ADAS)	44
12 m x 3 m	5 September 2016	No	Huntingdon, Cambs. (ADAS)	52
12 m x 3 m	29 August 2017	No	Huntingdon, Cambs. (ADAS)	46
4 m x 0.5 m	4 September 2015	Yes	Hinxton, Cambs. (NIAB)	44
4 m x 0.5 m	25 August 2017	Yes	Saxham, Suffolk (NIAB)	46
4 m x 0.5 m	23 August 2017*	Yes	Stowlangtoft, Suffolk (NIAB)	46

*replaces trial lost in autumn 2016

Plots were inspected from mid-June onwards in each year and symptoms assessed during late June or early to mid July. Thirty stems per plot were pulled up, and the middle to upper thirds of each stem scored for the presence of stem shredding and black microsclerotia. Stems were assigned to infection classes defined in Table 3. For analysis, an index (0-100) was calculated by multiplying the number of stems in each category by the numerical value of the category, dividing by the total number of stems, and multiplying by 100/5.

Table 3. Infection classes for severity of Verticillium symptoms on oilseed rape stems.

Scale point	Description
0	no symptoms
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

If no microsclerotia were visible, a section of stem was scraped back so that any sub-epidermal grey discolouration typical of Verticillium infection could be seen. If this was present, scores were revised to take it into account. Where stems did not show any visible sign of infection either externally or after scraping, a small sample was taken to test for the presence of Verticillium by plating onto agar. Sections of stem approximately 0.5 cm long were surface sterilised (7% sodium hypochlorite for 5 minutes) and placed directly onto potato dextrose agar plates. The presence of any *V. longisporum* colonies was recorded after 14 days incubation under 12 h uv/12 h dark. Sections from five clearly infected stems were plated as well to confirm presence of *V. longisporum*. A small subset of varieties was re-scored in the field approximately one week after the first score to determine whether any changes in rank order were observed as varieties matured. The percentage of pod senescence was assessed per plant visually, on the same destructive sample, with 100% being all pods yellow and senescent. Stem canker (*Leptosphaeria maculans*) developed in some trials, and was scored using Recommended List protocols to generate a canker index (0-100).

3.2 Yield experiments

Based on the results of screening experiments in 2016, six varieties with significantly different levels of symptom expression from low to high were selected for inclusion in yield experiments for harvest years 2017 and 2018 at two sites in each year. Site details are shown in Table 4. Trial design was a split plot, with main plots being either inoculated using the method described for screening experiments or left non-inoculated, and varieties were sub plots. There were four replicates. The experiments received a full crop protection programme of fungicides, herbicides and pesticides, including insecticides. At one site in each year, previous oilseed rape crops had been infected with Verticillium and inoculation was used to elevate infection levels further. At the other two sites, there had been no previous record of infection. All trials were assessed for symptom development in July using the method described for screening experiments, and then harvested using small plot combines. Oil content was determined by NMR and thousand seed weight was determined in one trial.

Table 4. Site details for yield experiments.

Plot size	Sowing date	Inoculation	Location	Previous disease level
12 m x 2 m	30 August 2016	Yes	Huntingdon, Cambs. (ADAS)	High
12 m x 2 m	21 September 2016	Yes	Higham, Suffolk (NIAB)	None
12 m x 2 m	29 August 2017	Yes	Stowlangtoft, Suffolk NIAB)	None
12 m x 2 m	25 August 2017	Yes	Huntingdon, Cambs (ADAS)	High

4. Results

4.1 Resistance screening experiments

There were statistically significant differences in the level of symptom development ($P < 0.05$) between varieties in all of the six screening experiments (see Figures 2, 3, 4, 5, 6 and 7, means are tabulated in the Appendix). The designated partially resistant and susceptible control varieties (shown as red bars) behaved as expected in all three years with lower infection levels on the resistant controls and consistently higher levels on the susceptible varieties Harper and Quartz, though the latter was unavailable in 2018.

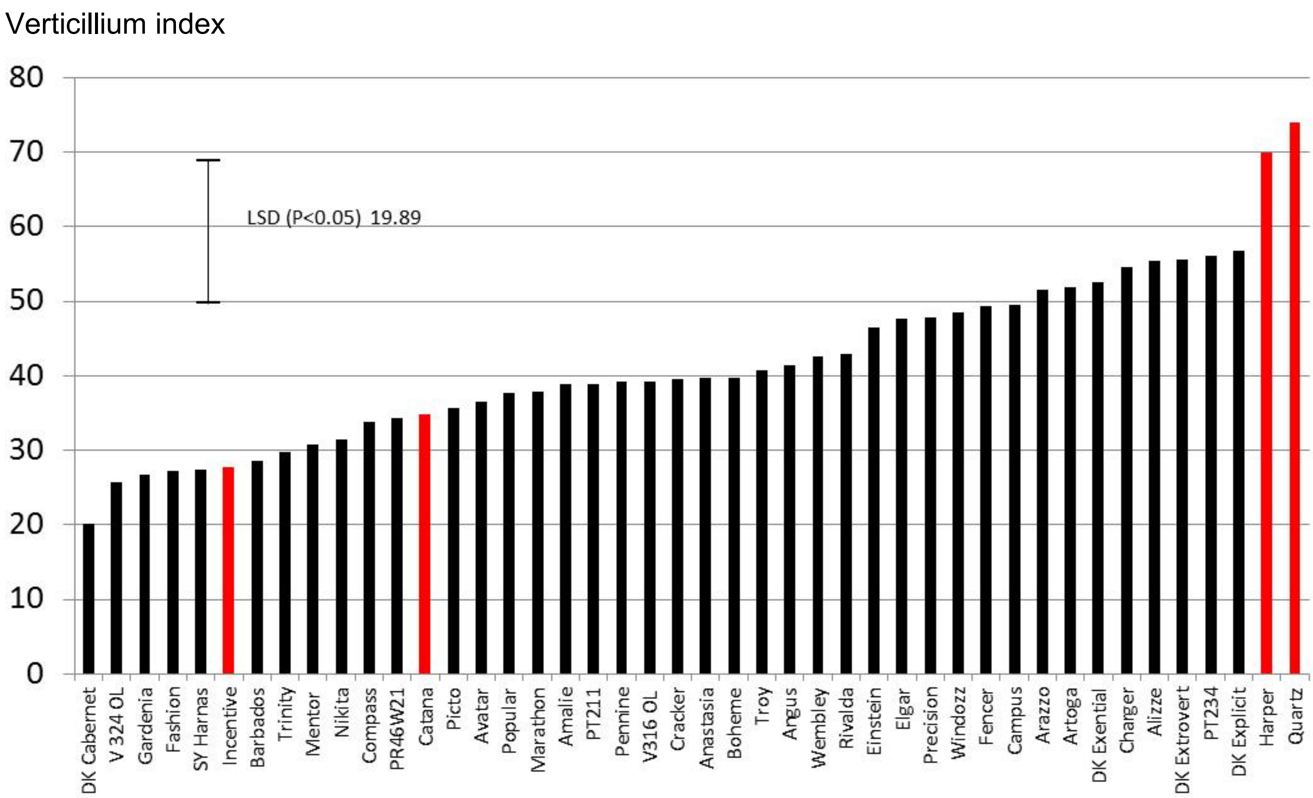

Figure 2. Verticillium indices, 2016 harvest year, natural infection ADAS trial. Red bars are the control varieties.

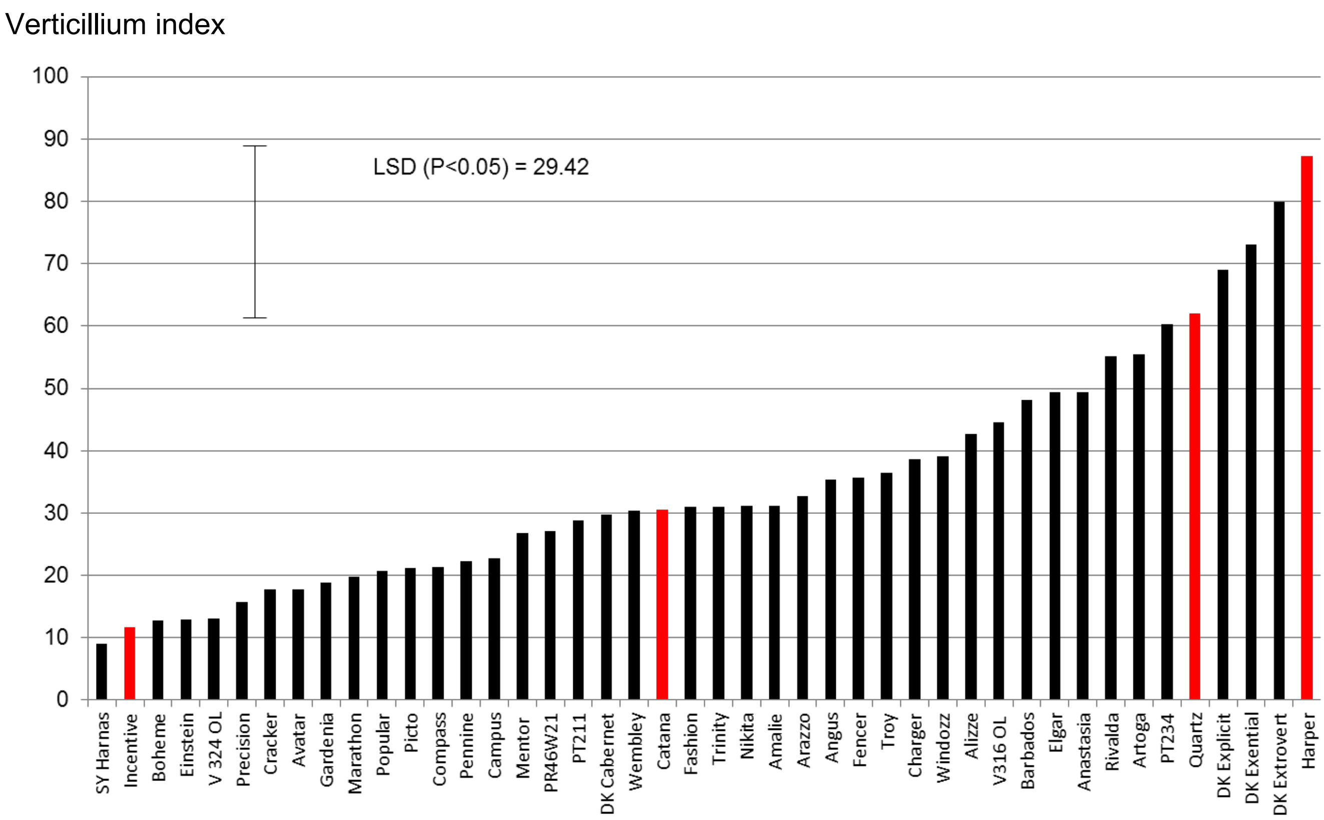

Figure 3. Verticillium indices, 2016 harvest year, inoculated NIAB trial. Red bars are the control varieties.

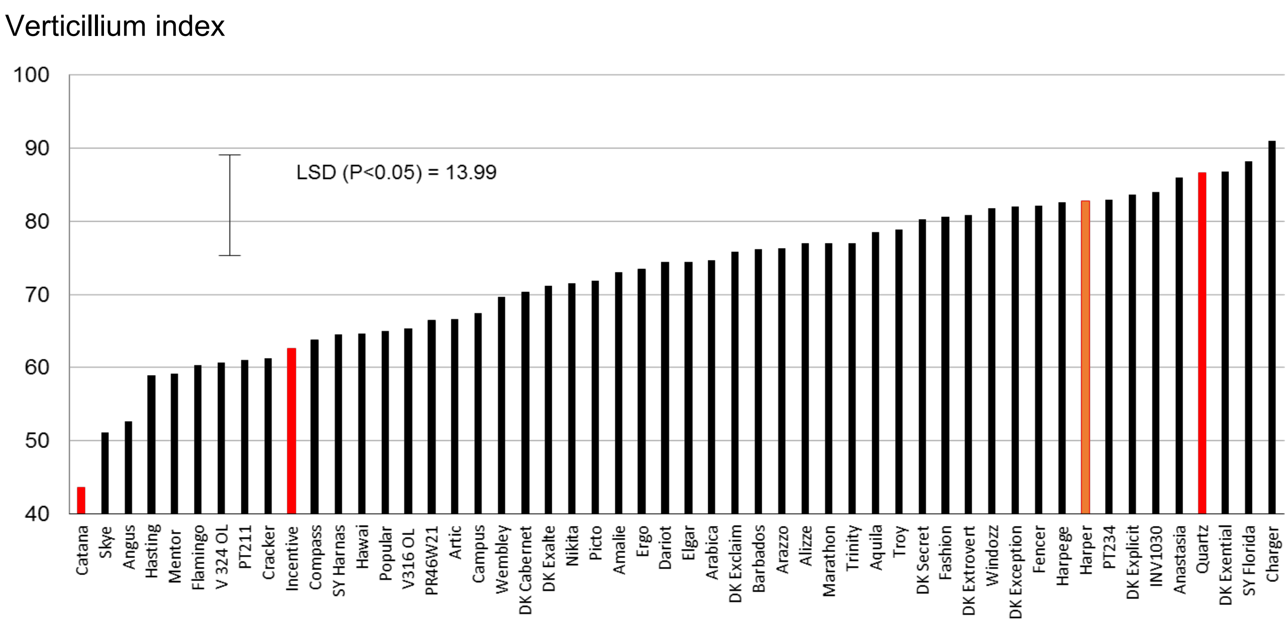

Figure 4. Verticillium indices, 2017 harvest year, natural infection ADAS trial. Red bars are the control varieties.

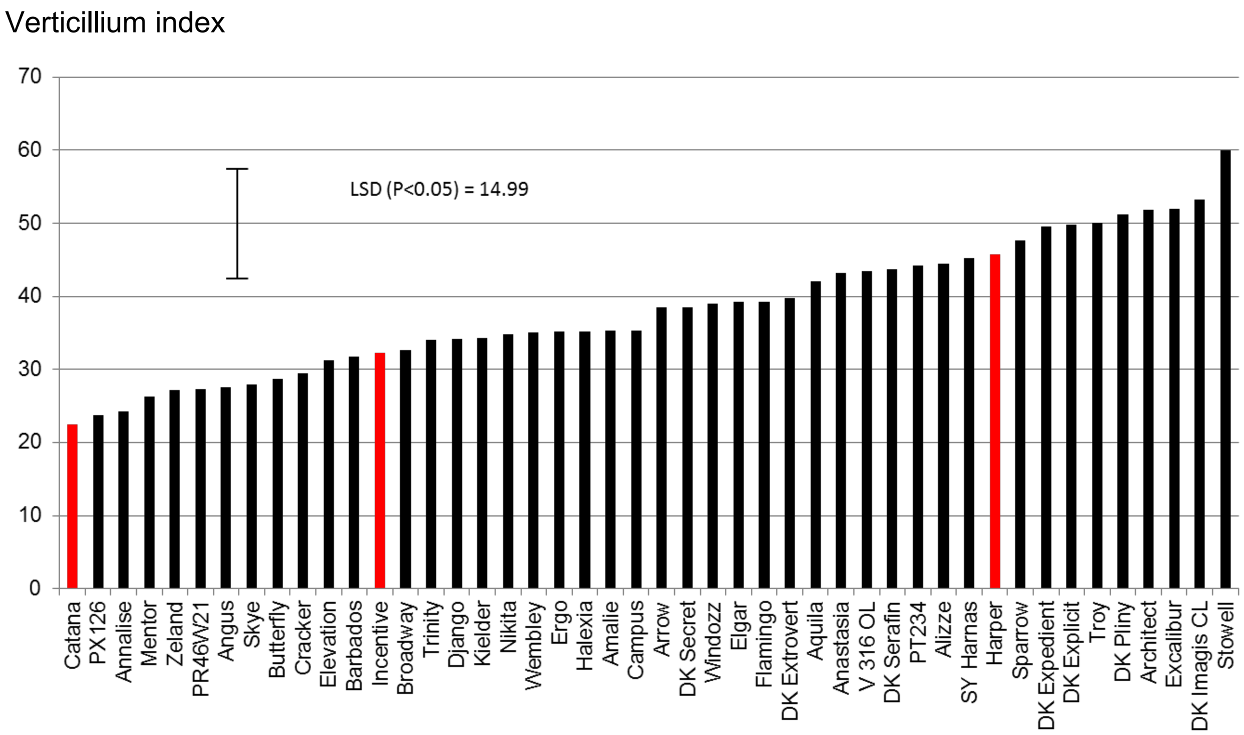

Figure 5. Verticillium indices, 2018 harvest year, natural infection ADAS trial. Red bars are the control varieties.

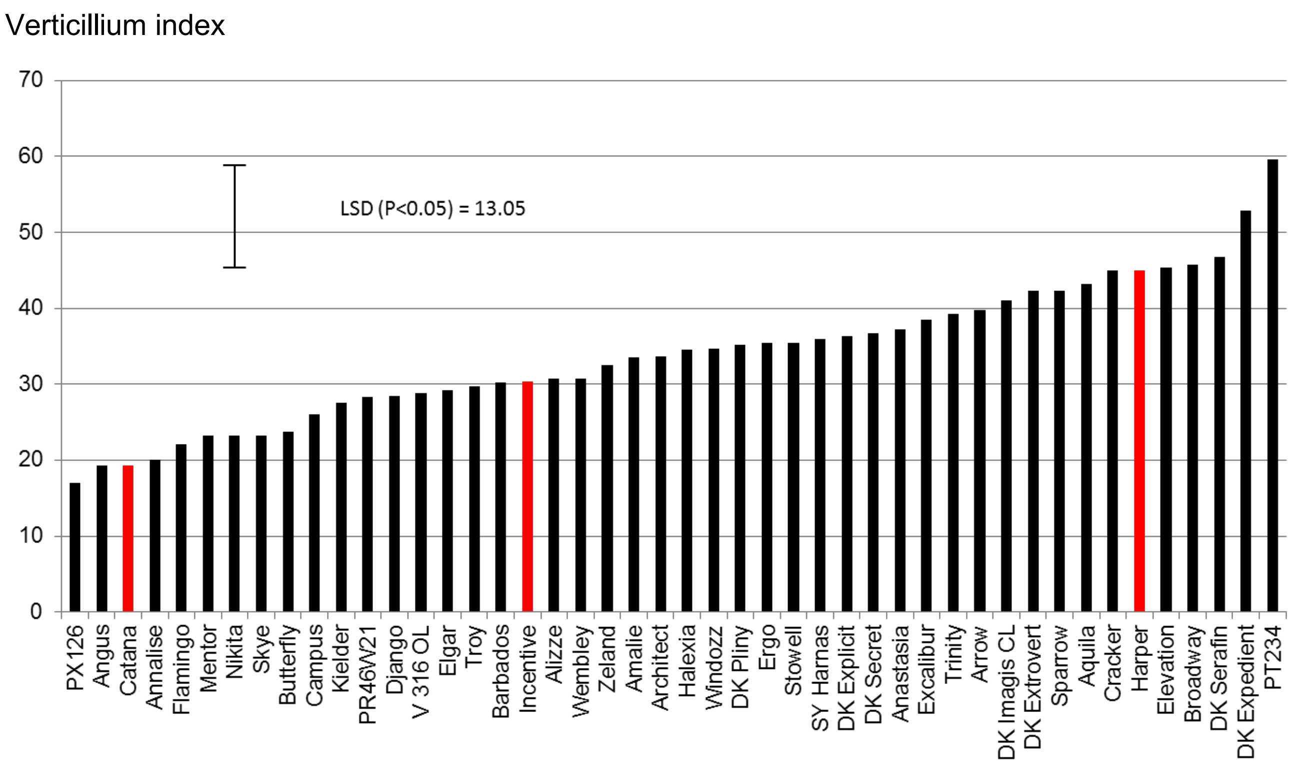

Figure 6. Verticillium indices, 2018 harvest year, inoculated NIAB trial (Saxham). Red bars are the control varieties.

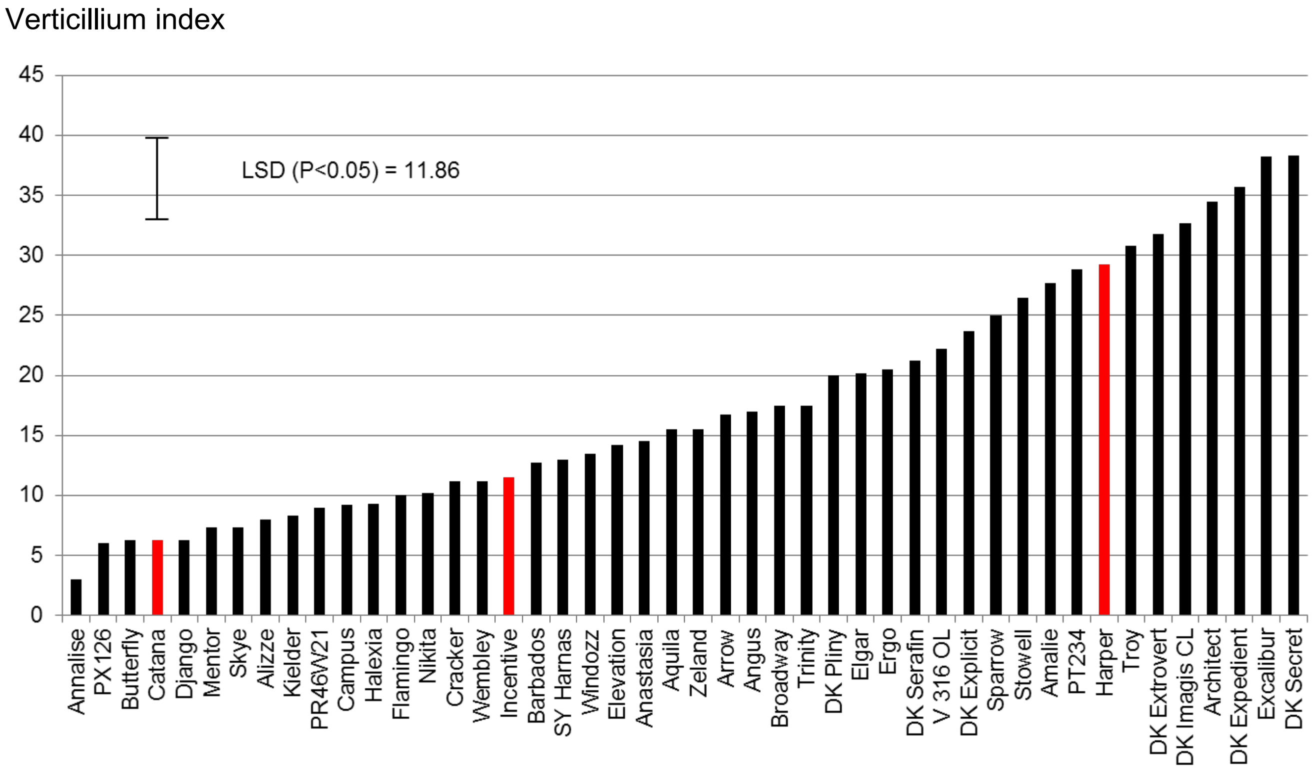

Figure 7. Verticillium indices, 2018 harvest year, inoculated NIAB trial (Stowlangtoft). Red bars are the control varieties.

Pod senescence showed a very strong positive relationship with Verticillium index in the 2016-harvested naturally infected ADAS experiment (Figure 8), but not at the inoculated NIAB site (Figure 9). In harvest 2017 there was also a strong near linear relationship at the naturally infected ADAS site (Figure 10). In 2018, the very hot conditions accelerated senescence at the two NIAB sites and the ADAS site, resulting in the pod canopy appearing very similar across all varieties at the time Verticillium was being scored, and scoring for differences was not feasible.

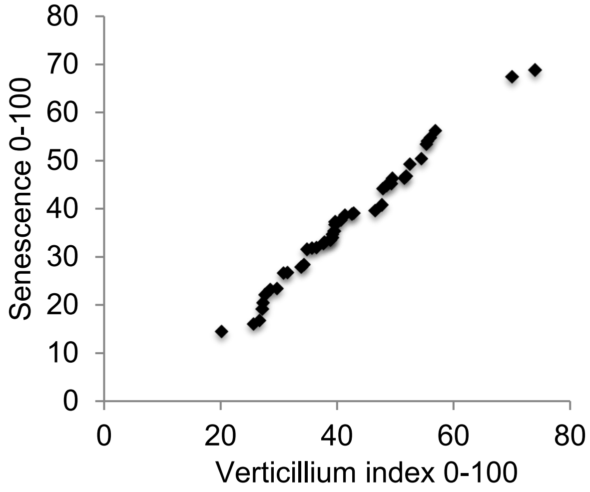

Figure 8. Relationship between senescence of the pod canopy and Verticillium index in the 2016-harvested naturally infected ADAS experiment.

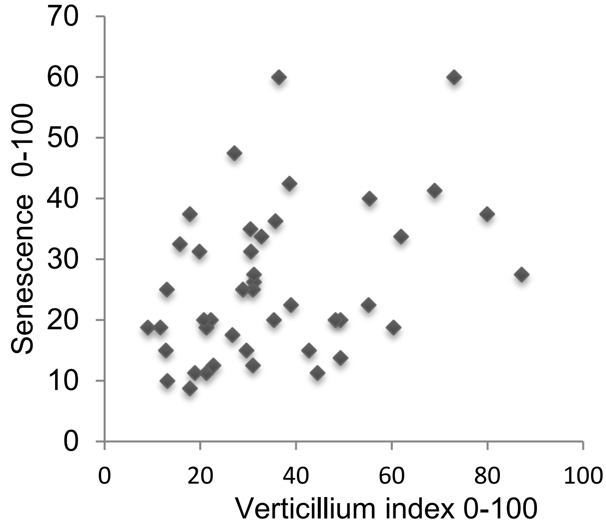

Figure 9. Relationship between senescence of the pod canopy and Verticillium index in the 2016-harvested inoculated NIAB experiment

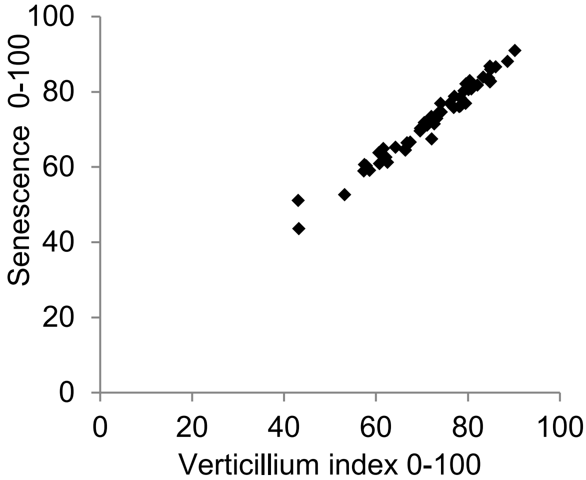

Figure 10. Relationship between senescence of the pod canopy and Verticillium index at the 2017 harvested naturally infected ADAS site.

Plating of stem sections which showed no Verticillium symptoms in the field did not give rise to any *V. longisporum* colonies, whereas those with either microsclerotia visible or sub-epidermal grey discolouration, always gave rise to colonies.

Re-scoring of variety subsets was possible in three of the six experiments. At the NIAB site in harvest 2016, the whole variety set was re-scored as initial results had been relatively low. Results showed that varieties ranked in the same order at both scoring times, though indices increased overall during a relatively short period (Figure 11). Rapid maturity precluded re-scoring at the two NIAB inoculated sites and the ADAS site in 2018. At the ADAS site in 2016, rank order at the second assessment on 20 July was similar to the first on 12 July, however, it was far more difficult to score the plots accurately at the second assessment. The situation was similar in 2017, with slightly lower scores at the later assessment for more resistant varieties and higher scores for more susceptible varieties. In both years, stems were far drier at the second assessment, which made scraping necessary to determine whether there were internal symptoms present, in the absence of external symptoms, (Table 5).

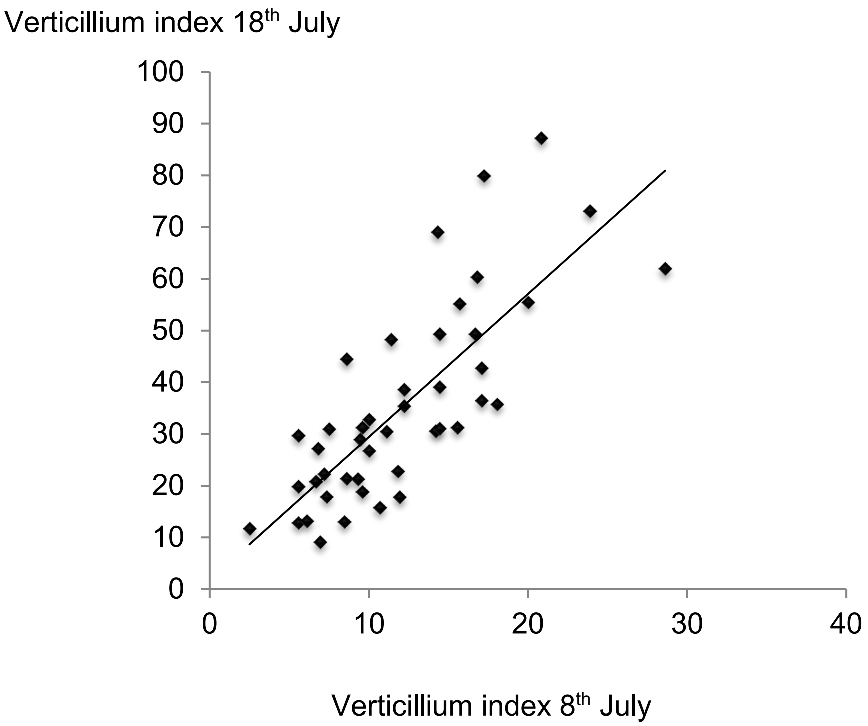

Figure 11. Relationship between Verticillium index variety scores separated by 10 days at the NIAB inoculated site, harvest 2016.

Table 5. Verticillium indices (0-100) from re-scoring of variety sub-sets in naturally infected ADAS trials in harvest 2016 and 2017.

Variety	ADAS site 12 July 2016	ADAS site 20 July 2016	ADAS site 12 July 2017	ADAS site 18 July 2017
V 324 OL	25.7	16.8	60.7	28.8
Incentive	27.7	15.2	62.7	37.5
Trinity	29.7	40.0	77.0	94.3
Catana	34.8	37.3	43.7	42.0
Elgar	-	-	74.5	100.0
Rivalda	42.8	41.8	-	-
Quartz	74.0	96.0	86.7	100.0
LSD p<05	19.89	60.00	13.99	32.45

There were significant (P<0.05) relationships between Verticillium scores between sites within a year, and between years for common sets of varieties. For a smaller sub-set of varieties included in all tests, there was a strong relationship between mean of all inoculated and all natural infection sites (Table 6).

Table 6. R^2 values for comparisons between sites, years and infection method.

R^2 coefficient	Comparison	Number of varieties in data set
46	Between sites 2016	44
40	Between years 2016 (2 trials) and 2017	39
62	Between years 2017 and 2018 (3 trials)	28
68	Between infection methods (3 trials each)	22

There was no significant association between Verticillium indices and stem canker indices at the ADAS site in 2016 (Figure 12), and a significant but weak association in 2017 (Figure 13).

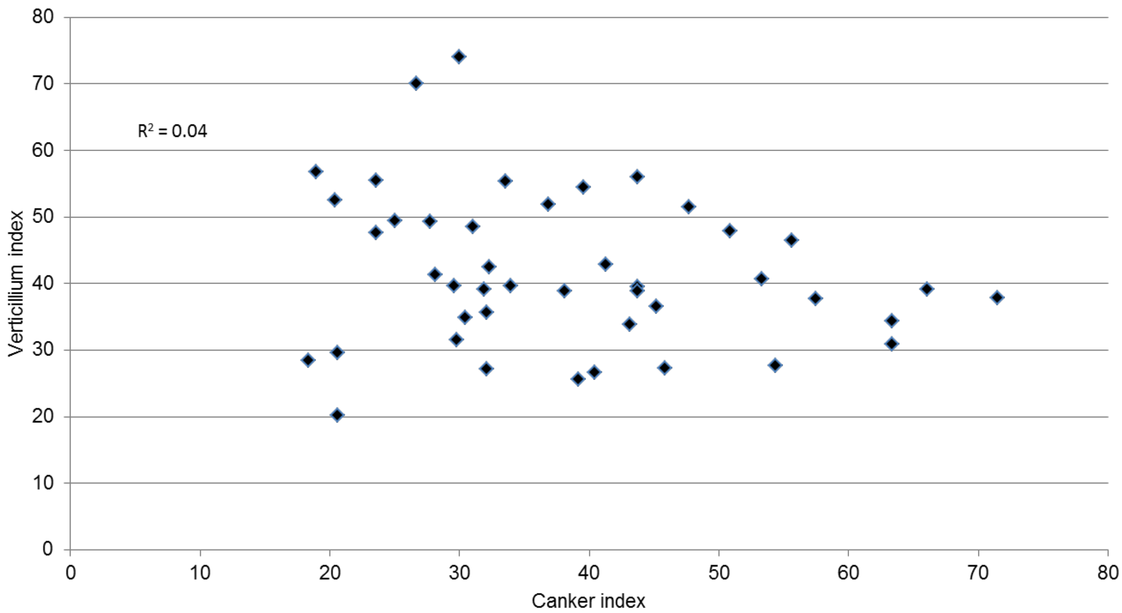

Figure 12. Relationship between Verticillium indices and stem canker indices for 44 oilseed varieties at the naturally infected ADAS site in 2016.

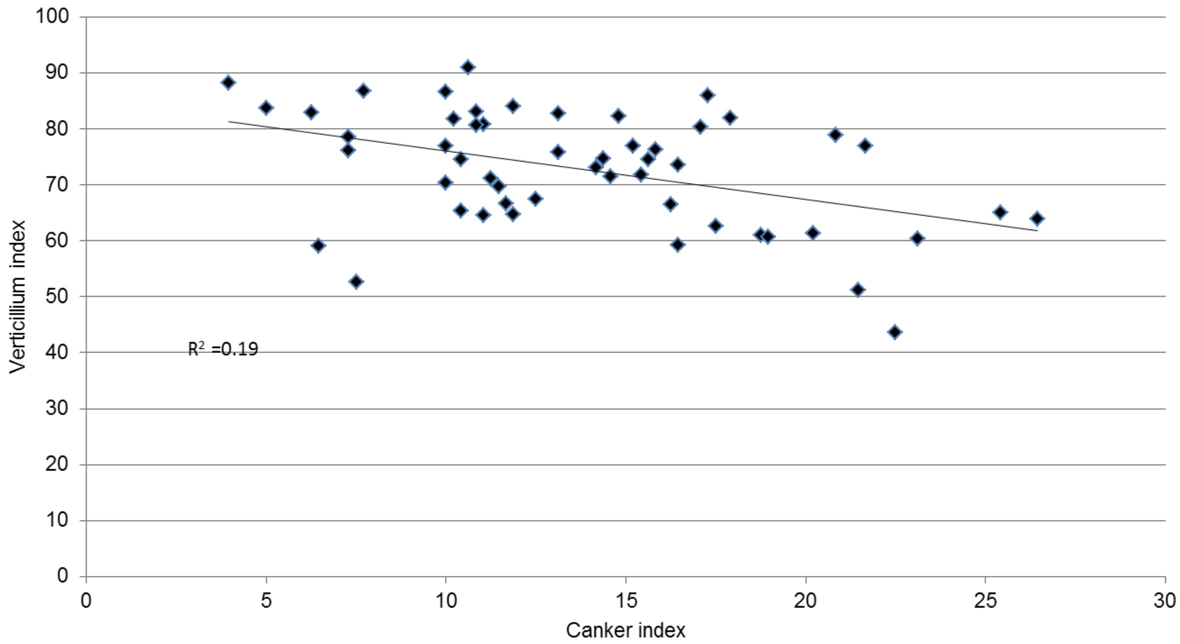

Figure 13. Relationship between Verticillium indices and stem canker indices for 52 oilseed varieties, at the naturally infected ADAS site in harvest 2017.

4.2 Yield experiments

Six varieties were selected on the basis of results from the harvest 2016 screening experiments to represent most resistant and most susceptible types (see Table 7 for summary of Verticillium indices). The same varieties were used for harvest 2017 and 2018 trials.

Table 7. Verticillium indices for six varieties selected for yield trials.

Variety	NIAB site 2016	ADAS site 2016	Mean
SY Harnas	9.1	27.3	18.2
Incentive	11.7	27.7	19.7
Catana	30.6	34.8	32.7
PT234	60.3	56.0	58.2
DK Extrovert	79.9	55.5	67.7
Harper	87.2	70.0	78.6
LSD (P<0.05)	29.44	19.89	

The selected varieties ranked as expected in all of the inoculated yield trials. In two trials (ADAS harvest 2017 and 2018) the level of background infection was high, and there was little differentiation in Verticillium indices between inoculated and non-inoculated blocks. In a third trial (NIAB, harvest 2017) where there was no previous history of disease, differences were more pronounced, though some infection was still seen in the non-inoculated blocks. In the fourth trial (NIAB harvest 2018) also where there was no previous history of disease, there was clear differentiation and very little disease developed on any variety in the non-inoculated blocks (Table 8).

Table 8. Verticillium indices on six varieties in inoculated (plus) and non-inoculated (minus) blocks in yield trials for harvest 2017 and 2018.

Variety	NIAB 2017 Inoculum level		ADAS 2017 Inoculum level		NIAB 2018 Inoculum level		ADAS 2018 Inoculum level	
	Plus	Minus	Plus	Minus	Plus	Minus	Plus	Minus
SY Harnas	38.8	21.3	49.5	42.7	12.3	1.0	27.5	30.0
Incentive	17.5	17.5	24.2	27.8	5.3	2.7	30.3	22.0
Catana	26.2	17.5	23.2	19.7	2.3	1.7	15.5	10.7
PT234	67.5	31.3	47.7	54.5	47.4	3.5	41.5	39.7
DK Extrovert	62.5	13.8	45.8	44.3	36.9	1.8	36.7	34.0
Harper	66.3	12.5	61.8	43.2	54.2	2.8	46.5	45.3

In the NIAB harvest 2018 yield trial, two sequential scores of Verticillium were taken, with a mean being used in Table 8. Rank orders were the same at each scoring occasion between the three susceptible and three resistant varieties (Table 9). Pod canopy senescence scores were taken at the ADAS sites in both years, again showing a strong positive relationship with the Verticillium index rankings (Table 10), though there was little difference between inoculated and non-inoculated scores.

Table 9. Verticillium indices (0-100) from re-scoring of varieties in NIAB 2018 yield trial.

Variety	Assessment date	
	4th July 2018	1st August 2018
SY Harnas	8.5	16.0
Incentive	10.2	0.5
Catana	4.5	0.0
PT234	18.5	76.3
DK Extrovert	17.7	56.3
Harper	30.8	77.5

Table 10. Pod senescence scores (0-100) for six varieties in inoculated (plus) and non-inoculated (minus) blocks in ADAS yield trials in 2017 and 2018.

Variety	ADAS 2017 Inoculum level		ADAS 2018 Inoculum level	
	Plus	Minus	Plus	Minus
SY Harnas	53.8	39.9	23.3	25.1
Incentive	26.9	32.4	35.0	18.6
Catana	20.9	16.5	10.3	5.8
PT234	49.3	56.7	38.8	38.6
DK Extrovert	46.1	45.3	35.4	34.6
Harper	61.3	45.3	49.0	45.5

Yield was significantly reduced (P<0.05) in inoculated blocks compared to non-inoculated in one trial (NIAB 2018, with no previous history of disease). Two of the more susceptible varieties (PT234 and Harper) showed the greatest yield reduction, while DK Extrovert showed a smaller, non-significant reduction even though disease levels in this variety were high in the inoculated blocks. No significant yield reductions were seen in partially resistant varieties. In the NIAB 2017 trial, where some disease was recorded in non-inoculated blocks, there was still a tendency for the more susceptible varieties PT234 and Harper to show a greater yield reduction (non-inoculated block yield less inoculated block yield) compared to the partially resistant varieties, though reductions were not significant. Again disease had no effect on the yield of DK Extrovert (Table 11).

Table 11. Yield (t ha^{-1}) for six oilseed rape varieties with differing levels of resistance to Verticillium in inoculated (plus) and non-inoculated (minus) plots.

Variety	NIAB 2017 Inoculum level		ADAS 2017 Inoculum level		NIAB 2018 Inoculum level		ADAS 2018 Inoculum level	
	Plus	Minus	Plus	Minus	Plus	Minus	Plus	Minus
SY Harnas	3.12	3.00	2.01	2.02	4.02	4.16	3.75	3.86
Incentive	3.14	3.27	2.22	2.16	3.71	3.83	4.08	3.83
Catana	3.08	2.94	1.60	1.56	3.74	3.63	3.54	3.57
PT234	3.36	3.62	2.15	1.76	3.87	4.56	3.74	3.71
DK Extrovert	3.22	3.17	2.08	1.89	3.91	4.13	3.88	4.00
Harper	3.35	3.78	2.09	2.29	3.92	4.37	3.88	3.71
LSD (*P*<0.05) (variety x inoculum)	0.523 (ns)		0.499 (ns)		0.421		0.926 (ns)	

There were no significant effects of inoculum level or variety on oil content in either ADAS trial in 2017 and 2018. There was a significant effect of variety in both NIAB trials, but no effect of inoculum level (Table 12). Thousand seed weight was measured in the NIAB 2018 trial where significant yield effects were observed, but there was no effect of inoculum or variety (Table 13).

Table 12. Oil content (%) for six oilseed rape varieties with differing levels of resistance to Verticillium in yield trials in inoculated (plus) and non-inoculated (minus) plots.

Variety	NIAB 2017 Inoculum level		ADAS 2017 Inoculum level		NIAB 2018 Inoculum level		ADAS 2018 Inoculum level	
	Plus	Minus	Plus	Minus	Plus	Minus	Plus	Minus
SY Harnas	45.2	44.9	43.3	43.2	42.8	42.7	42.7	41.4
Incentive	46.4	45.8	44.9	44.7	43.8	43.6	45.1	44.9
Catana	47.1	47.1	44.0	45.6	45.4	45.6	44.9	45.1
PT234	47.0	46.4	45.3	44.6	44.4	45.2	44.2	44.5
DK Extrovert	46.5	46.3	45.6	45.8	44.8	44.6	45.2	44.8
Harper	46.5	45.7	44.1	44.5	43.9	44.4	43.4	44.3
LSD *(P*<0.05)								
Variety	0.54		0.52 (ns)		0.62		0.34 (ns)	
Inoculum level	0.79 (ns)		1.52 (ns)		1.32 (ns)		1.43 (ns)	

Table 13. Thousand seed weight (g) for six oilseed rape varieties with differing levels of resistance to Verticillium in the NIAB 2018 yield trial in inoculated (plus) and non-inoculated (minus) plots.

Variety	NIAB 2018 Inoculum level	
	Plus	Minus
SY Harnas	6.4	7.0
Incentive	6.4	6.6
Catana	6.7	7.0
PT234	5.6	5.7
DK Extrovert	5.9	6.1
Harper	6.5	6.6

5. Discussion

Results from this project confirm that there are consistent differences between Recommended List oilseed rape varieties in the severity of Verticillium symptoms. Both the inoculated test method and natural infection method produced broadly similar results, and both would be suitable for future test programmes, provided that naturally infected sites are sufficiently uniform in disease distribution. In this project, previous knowledge of uniform symptom severity in a preceding oilseed rape crop was available. Inoculation can be accurately quantified in terms of colony forming units applied to unit area, whereas natural infection may be locally variable within fields. Knowledge of previous infection patterns is necessary to avoid this and place trials in areas of uniform infection. Isolates used in the inoculated tests originated from infected crops in Suffolk and Lincolnshire and were confirmed as the A1D1 lineage (Depotter *et al*., 2017). The strong relationship between inoculated tests, and natural infection trials at different locations, indicates that the *V. longisporum* population in the UK is uniform, i.e. there are no discernible population x variety interactions, and ratings are likely to be stable.

Re-scoring of sub-sets of varieties, whether from the screening experiments or yield trials, showed that rank order was always preserved as varieties matured, thus more resistant varieties never expressed disease levels equivalent to more susceptible ones even at late stages of development before harvest. Timing of scoring was however critical. Symptoms developed rapidly over a period of 7 to 14 days, and scoring too early could mean under-estimation of symptom incidence and severity, even with scraping to reveal sub-epidermal symptoms. Equally, scoring too late could mean difficulty in seeing Verticillium on stems which are rapidly drying out. It was noted during the re-scoring at ADAS sites that, although late assessment of disease incidence and severity was possible, it took much longer due to the dryness of the stems. Inspection of trials every three days around end of June to early July is recommended to judge the correct time for scoring.

The stem scoring system devised in the project was objective, and enabled different scorers to maintain comparability. The relationships between scores from different sites (and therefore different scoring teams) were at least as good, and sometimes better, than those seen for stem canker assessments in Recommended List trials. Despite using autumn sprays to control stem canker in the Verticillium screening trials, canker did develop in some trials, and was assessed. The absence of any strong relationship between canker and Verticillium indices on the same plots illustrates the robustness of the scoring systems. Additionally the observation, at least with the variety sets used, suggests that canker resistance is not linked to Verticillium susceptibility, and *vice versa*. However, it was still apparent that several varieties with high canker resistance (presumed to be carrying RLm7) do tend to be more susceptible to Verticillium.

Pod canopy senescence proved a very good predictor of Verticillium index, and could be considered as a proxy for recording disease symptoms. However, this relationship was only seen in larger (12 m x 2 m) plots. Smaller variety screening plots had relatively open canopies, and a high proportion of wheeling and pathway area to plot area, causing relatively rapid senescence which likely masked disease effects on the pod canopy. However, any future evaluation system which uses pod canopy senescence as an indicator of Verticillium resistance rather than the labour intensive stem scoring system should still ensure that Verticillium symptoms are present. The assessment of pod canopy senescence still had to be carried out on destructively sampled plants to get this relationship. Scoring pod senescence scores caused by Verticillium using non-destructive whole plot methods does not result in a similar score (Ritchie, unpublished data). Checks on control varieties in particular should be used to verify association between pod senescence and disease in individual trials.

None of the varieties tested was completely resistant, but some showed low levels of infection even under relatively high disease pressure. This suggests that variety selection could form an effective means of reducing infection and the subsequent return of microsclerotia to the soil. Where fields are thought to have low levels of infection, selection of a partially resistant variety should help to prevent further increase. There were several varieties with resistance levels similar to, or better than, the now outclassed variety Catana, so a choice of material is likely to be available. Future breeding for elevated resistance should be possible from elite material.

Yields of varieties with high levels of disease tended to be reduced more than those with low levels where inoculation had created clear differences in symptom expression compared to heathy plots. There was an indication that some varieties such as DK Extrovert may be tolerant to infection, having a small and non-significant yield penalty despite high infection levels. The existence of tolerant varieties was also noted by Gladders *et al*., (2013). Yield losses in the susceptible PT234 and Harper were 15% and 10% respectively in one trial. The more resistant Incentive only incurred a 3% loss which was not significant. However, oil content was not significantly affected by the presence of infection and, surprisingly, thousand seed weight was unaffected in the trial which showed significant yield depression. This indicates that components of yield such as pod number or seeds per pod were being affected, rather than seed size. In common with other reports on the yield effects of Verticillium, there was considerable variability in the experiments reported here. The effects of site and season on yield potential will likely influence the impact of Verticillium. However, differential effects on yield depending on variety susceptibility have been observed both in this study, and that of Depotter *et al.*, (2018). The latter work was conducted in the UK, using inoculated and non-inoculated blocks of Incentive, Vision, Harper and Quartz. Significant yield losses were incurred in all varieties in one year (2016), but not in 2017. Losses were greater in Quartz (34%), and Harper (15%) than in Incentive (11%). This suggests that the range of variety resistance observed is relevant, and more resistant ones can be deployed to avoid large yield penalties where fields have become infected. Variety resistance ratings, if available through the Recommended List, would therefore be relevant information for growers.

Ratings may be based on the standard 1-9 scale, or a less precise ranking depending on the quantity and quality of future data from either inoculated tests or trials located in areas of high natural infection. An over-trials analysis of the variety set which occurred in all six tests has been carried out. Control settings were selected based on using a “top three and bottom three” average and calculating ratings on a 1 to 9 scale using the log data. A range from 7.6 (Catana) to 2.1 (Harper) was obtained (see Table in Appendix). Using an LSD between variety means to compare calculated rating points indicated that a rating point difference of just under 2.0 would be significant at $P<0.05$. Whether this approach is used to provide information to growers, or a less differentiated scale such as A to D where A would signify high resistance and D very susceptible, depends on future data collection. Currently, there is no National List testing for Verticillium resistance, unlike stem canker and light leaf spot. There is therefore no preliminary data on varieties entering RL candidate trials. Results from this project would indicate at least two independent data points during candidate trialling would be able to give a good initial prediction of Verticillium resistance. Further repeat testing at RL would consolidate data, and a target of six datapoints would likely give the level of precision seen in this project. For those varieties already on the 2018/19 Recommended List, Mentor and SY Harnas offer moderate to good resistance, based on data from the subset of varieties which occurred in all six tests.

This project, together with other recent and ongoing research, has considerably advanced knowledge of *Verticillium longisporum* biology, pathogenicity, host resistance and epidemiology. However, there is still very significant scope for further work which would aid growers in managing the impact of the pathogen. More investigation of the nature of variety tolerance is needed, and ongoing evaluation of the yield effects of Verticillium on newer varieties. Establishing the relationship between levels of disease inoculum in soil, and impact on productivity, is an essential next step. Understanding field risk level would aid growers in variety selection. While a quantitative soil diagnostic has been developed by several groups, interpretation is limited without fuller understanding of the inoculum levels likely to cause yield losses. Breeding efforts to increase at least partial resistance to the pathogen are ongoing, and the relatively high levels of resistance seen in varieties such as Catana indicate that improvements should be possible from elite material.

6. References

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Appendix

Mean Verticillium indices (0-100) on 44 oilseed rape varieties at two sites in July 2016.

Variety	ADAS not inoculated	NIAB inoculated
Alizze	55.3	42.7
Amalie	38.8	31.2
Anastasia	39.7	49.3
Angus	41.3	35.4
Arazzo	51.5	32.8
Artoga	51.8	55.4
Avatar	36.5	17.8
Barbados	28.5	48.2
Boheme	39.7	12.8
Campus	49.5	22.8
Catana	34.8	30.6
Charger	54.5	38.6
Compass	33.8	21.3
Cracker	39.5	17.8
DK Cabernet	20.2	29.7
DK Exential	52.5	73.1
DK Explicit	56.8	69.0
DK Extrovert	55.5	79.9
Einstein	46.5	13.0
Elgar	47.7	49.3
Fashion	27.2	30.9
Fencer	49.3	35.7
Gardenia	26.7	18.8
Harper	70.0	87.2
Incentive	27.7	11.7
Marathon	37.8	19.8
Mentor	30.8	26.7
Nikita	31.5	31.2
Pennine	39.2	22.2
Picto	35.7	21.3
Popular	37.7	20.8

PR46W21	34.3	27.1
Precision	47.8	15.8
PT211	38.8	28.9
2016 continued		
PT234	56.0	60.3
Quartz	74.0	61.9
Rivalda	42.8	55.1
SY Harnas	27.3	9.1
Trinity	29.7	31.0
Troy	40.7	36.4
V 324 OL	25.7	13.1
V316 OL	39.2	44.5
Wembley	42.5	30.4
Windozz	48.5	39.0
LSD P<0.05	19.89	29.42

Mean Verticillium indices (0-100) on 52 varieties of oilseed rape at a single site*,July 2017.

Variety	ADAS not inoculated
Alizze	77.0
Amalie	73.0
Anastasia	86.0
Angus	52.7
Aquila	78.5
Arabica	74.7
Arazzo	76.3
Artic	66.7
Barbados	76.2
Campus	67.5
Catana	43.7
Charger	91.0
Compass	63.8
Cracker	61.3
Dariot	74.5
DK Cabernet	70.3
DK Exalte	71.2
DK Exception	82.0
DK Exclaim	75.8

DK Exential	86.8
DK Explicit	83.7
DK Extrovert	80.8
2017 continued	
DK Secret	80.3
Elgar	74.5
Ergo	73.5
Fashion	80.7
Fencer	82.2
Flamingo	60.3
Harpege	82.7
Harper	82.8
Hasting	59.0
Hawai	64.7
Incentive	62.7
INV1030	84.0
Marathon	77.0
Mentor	59.2
Nikita	71.5
Picto	71.8
Popular	65.0
PR46W21	66.5
PT211	61.0
PT234	83.0
Quartz	86.7
Skye	51.2
SY Florida	88.2
SY Harnas	64.5
Trinity	77.0
Troy	78.8
V 324 OL	60.7
V316 OL	65.3
Wembley	69.7
Windozz	81.8
LSD $P<0.05$	13.99

*NIAB inoculated site failed due to cabbage stem flea beetle

Mean Verticillium indices (0-100) on 46 varieties of oilseed rape at three sites*, June/July 2018.

Variety	NIAB (Saxham) Inoculated	NIAB (Stowlangtoft) Inoculated	ADAS not inoculated
Alizze	30.7	8.0	44.5
Amalie	33.5	27.7	35.3
Anastasia	37.2	14.5	43.2
Angus	19.3	17.0	27.5
Annalise	20.0	3.0	24.3
Aquila	43.2	15.5	42.0
Architect	33.7	34.5	51.8
Arrow	39.8	16.7	38.5
Barbados	30.2	12.7	31.8
Broadway	45.7	17.5	32.7
Butterfly	23.8	6.3	28.7
Campus	26.0	9.2	35.3
Catana	19.3	6.3	22.5
Cracker	45.0	11.2	29.5
Django	28.5	6.3	34.2
DK Expedient	52.8	35.7	49.5
DK Explicit	36.3	23.7	49.8
DK Extrovert	42.3	31.8	39.8
DK Imagis CL	41.0	32.7	53.2
DK Pliny	35.2	20.0	51.2
DK Secret	36.7	38.3	38.5
DK Serafin	46.8	21.2	43.7
Elevation	45.3	14.2	31.3
Elgar	29.2	20.2	39.3
Ergo	35.5	20.5	35.2
Excalibur	38.5	38.2	52.0
Flamingo	22.1	10.0	39.3
Halexia	34.5	9.3	35.2
Harper	45.0	29.2	45.8
Incentive	30.3	11.5	32.2
Kielder	27.5	8.3	34.3

Mentor	23.2	7.3	26.3
Nikita	23.2	10.2	34.8
2018 continued			
PR46W21	28.3	9.0	27.3
PT234	59.6	28.8	44.2
PX126	17.0	6.0	23.8
Skye	23.2	7.3	28.0
Sparrow	42.3	25.0	47.7
Stowell	35.5	26.5	60.0
SY Harnas	36.0	13.0	45.2
Trinity	39.3	17.5	34.0
Troy	29.7	30.8	50.0
V 316 OL	28.8	22.2	43.5
Wembley	30.7	11.2	35.0
Windozz	34.7	13.5	39.0
Zeland	32.5	15.5	27.2
LSD P<0.05	13.05	11.86	14.90

*additional inoculated NIAB site to replace CSFB loss in 2017

Summary of full matrix data set – log analysis and calculated 1-9 ratings (where 1 is very susceptible and 9 is very resistant).

Variety	Trial count	Mean Verticillium index (0-100)	Log index	Rating
Alizze	6	43.0	1.57	4.8
Amalie	6	39.9	1.59	4.5
Anastasia	6	45.0	1.61	4.1
Angus	6	32.2	1.49	5.9
Barbados	6	37.9	1.53	5.3
Campus	6	35.0	1.49	5.9
Catana	6	26.2	1.37	7.6
Cracker	6	34.0	1.48	6.0
DK Explicit	6	53.7	1.70	2.8
DK Extrovert	6	55.0	1.72	2.5
Elgar	6	43.4	1.61	4.1
Harper	6	60.0	1.76	2.1
Incentive	6	29.3	1.41	7.0
Mentor	6	28.9	1.41	7.0
Nikita	6	33.7	1.48	6.1
PR46W21	6	32.1	1.46	6.4
PT234	6	55.3	1.73	2.4
SY Harnas	6	32.5	1.44	6.6
Trinity	6	38.1	1.55	5.0
Troy	6	44.4	1.63	3.8
V316 OL	6	40.6	1.60	4.4
Wembley	6	36.6	1.52	5.4
Windozz	6	42.8	1.59	4.5
LSD (P<0.05)		9.60		