

Measuring the impact of *Verticillium longisporum* on oilseed rape (*Brassica napus*) yield in field trials in the United Kingdom

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Abstract *Verticillium longisporum* causes Verticillium stem striping on oilseed rape, which appears towards the end of the cropping season. Thus far, the impact of *V. longisporum* infection on oilseed yield remains unclear. In this study, we assessed the impact of Verticillium stem striping on British oilseed rape production. To this end, four cultivars (Incentive, Vision, Harper and Quartz) were grown in field plots with different levels of *V. longisporum* disease pressure at different locations over two consecutive years. Whereas Incentive and Vision developed relatively few stem striping symptoms, Harper and especially Quartz showed severe symptoms during these field experiments. Furthermore, higher inoculum levels induced more severe symptoms in these cultivars. Significant yield reductions upon *V. longisporum* infection only occurred in a single field trial on all tested oilseed rape cultivars. These preliminary data suggest that Verticillium stem striping does not consistently impact oilseed rape yield, despite the occurrence of abundant disease symptoms.

Keywords Verticillium stem striping · Yield loss · Disease development · Oil content

Verticillium stem striping is an oilseed rape disease caused by the fungal pathogen *Verticillium longisporum*, one of the ten species of the *Verticillium* genus (Depotter et al. 2016; Inderbitzin et al. 2011). This disease is well established in north-central Europe (Heale and Karapapa 1999; Steventon et al. 2002) and recently emerged in the United Kingdom for the first time (Depotter et al. 2017b; Gladders et al. 2011). In contrast to other *Verticillium* spp., *V. longisporum* generally infects brassicaceous plants (Inderbitzin and Subbarao 2014; Novakazi et al. 2015; Zeise and von Tiedemann 2002). On these hosts, *V. longisporum* causes wilting symptoms (Koike et al. 1994). However, no wilting symptoms are observed on oilseed rape (Heale and Karapapa 1999). Rather, dark unilateral striping is induced at the end of the cropping season before the onset of maturation, approximately 3–4 weeks before harvest. This coincides with the formation of black microsclerotia by the fungus in the stem cortex underneath the epidermis. There is no effective fungicide treatment to combat *Verticillium* diseases; hence, using resistant cultivars is the preferred management strategy against Verticillium stem striping (Depotter et al. 2016). Unfortunately, genuine resistance (*R*) genes are unavailable against *V. longisporum*. Alternatively, research efforts have been undertaken to improve partial *Verticillium* resistance in oilseed rape (Eynck et al. 2009; Obermeier et al. 2013). Despite these efforts to improve

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resistance in oilseed rape cultivars, limited studies investigated the actual impact of *Verticillium* stem striping on crop quality and quantity (Dunker et al. 2008). Yield losses by *Verticillium* stem striping have been anticipated to range from 10 to 50%, yet experimental verifications of such estimations are lacking (Dunker et al. 2008). In contrast, *Verticillium* stem striping did not impact yield significantly in previous field studies despite the presence of stem striping symptoms (Dunker et al. 2008).

To investigate the impact of *Verticillium* stem striping on oilseed rape production, we performed field experiments in two consecutive years at different locations in the United Kingdom (UK). In harvest year 2016, field plots were located in Hinxton, Cambridgeshire, UK, whereas in harvest year 2017 fields were situated in Higham, Suffolk, UK. Previously, winter oilseed rape cultivars were screened for their resistance against *Verticillium* stem striping under field conditions (not published). We selected four commercially used cultivars of this screen for our field experiments: two open-pollinated cultivars Vision (Senova) and Quartz (KWS), and two hybrid types: Incentive (DSV) and Harper (Bayer). In the screen, Incentive and Vision displayed little symptoms, whereas Harper and Quartz displayed stem-striping symptoms abundantly. These differences in susceptibility were also confirmed by pathogenicity tests under controlled greenhouse conditions, where Quartz displayed more severe disease symptom development upon *V. longisporum* inoculation compared to Incentive (Depotter et al. 2017a). The field plots were sown 5th and 21st September, for the field trials at Hinxton and Higham, respectively. To monitor putative dosage effects, plots were inoculated with different quantities of *V. longisporum* inoculum. To this end, a mix of British *Verticillium* strains were cultured in bags containing a sterile, moist medium of vermiculite (800 ml) and maize meal (26 g) for approximately four weeks and then dried at room temperature for a week prior to inoculation. The plots were inoculated by manually spreading 0.25 l or 2.5 l of thoroughly mixed, colonized vermiculite/maize meal medium on the soil surface. To enable homogenous dispersion, the 0.25 l of inoculum was first mixed with 0.75 l sand. A control treatment was left un-inoculated. Inoculations were performed the 16th of September 2015 for the field trial at Hinxton and the 26th of September 2016 for the trial at Higham. As four different cultivars and three different inoculum levels were tested in four replicates, the field

trial comprised of 48 plots, which were arranged in a semi-randomized block design and were 24 m² (12 m × 2 m) in size. The trials were managed according common agronomic practices with one fertilizer application in August (diammonium phosphate) and two in February–April (N + S and N, respectively). No fungicides and a standard insecticide programme were used (to control flea beetle during establishment and pollen beetle during flowering). Field plots were harvested on 31st and 26th July for the field trials at Hinxton and Higham, respectively. To this end, a central strip of 18 m² was harvested using a combine and used for analysis.

We assessed the *Verticillium* stem striping disease symptoms by monitoring severity and incidence. Disease symptom severity was measured based on the fraction of stem circumference that displayed *Verticillium* stem striping symptoms. For every plot at each time point, 20 stems were randomly chosen and scored on a 0–10 scale, with each interval representing a 10% increase in dark unilateral striping of the stem circumference at knee-height (eg. Score 2 = 20% striping). Disease incidence was the fraction of the stems containing disease symptoms with a minimal score of 1. The symptoms were scored four times each year starting from the end of June with a 7-day interval with the first assessment on 30th June and 27th June for year 2016 and 2017, respectively. In this way, area under disease progress curve (AUDPC) could be calculated by trapezoidal integration to compare disease severities over time (Shaner and Finney 1977).

In correspondence to the unpublished cultivar screen, cultivars Incentive and Vision displayed equally few disease symptoms at the end of both cropping seasons (Table 1). Consequently, little to no progress in disease symptoms was monitored in both years (Fig. 1). In contrast, Harper displayed more extensive disease symptoms in both consecutive trial years (Table 1). Altogether, disease symptom development was similar in harvest years 2016 and 2017 (Fig. 1). Higher inoculum levels resulted in increasing incidence and symptom severity in Harper for the 2016 trial (Table 1). However, whereas disease levels of the inoculated plots were higher than in control plots, symptoms levels between the lower and higher inoculum treatments could not be distinguished in 2017 (Table 1). Furthermore, Quartz displayed the most severe disease symptoms of the four tested cultivars upon *V. longisporum* challenge (Table 1). The area under disease progress curves

Table 1 Impact of *Verticillium* stem striping on oilseed rape: disease symptoms, yield and oil content. Data of disease incidence and symptom severity were assessed on 21 July and 18 July for year 2016 and 2017, respectively. Lower case letters indicate significant differences between cultivars in the same year, whereas capital letters indicate significant differences between cultivars of the same treatment in the same year. The treatment abbreviations “CTR”, “LOW” and “HIGH”, represent the data for plots were no, lower and higher amounts of *V. longisporium* inoculum were applied, respectively

Cultivar	Treatment	Disease incidence (%) ^a			Disease symptom severity (0–10) ^b			Yield (ton/ha) ^c			Oil content (%) ^d									
		2016	2017	2016	2016	2017	2016	2016	2017	2016	2016	2017								
Incentive	CTR	6.3	3.1	aA	3.8	2.4	aA	0	[0,0]	abA	3.23	0.08	a	3.24	0.08	a	46.3	a	48.9	a
	LOW	10.0	2.0	aA	10.0	2.0	aA	0	[0,0]	abA	3.03	0.12	ab	3.48	0.14	a	45.8	a	48.4	a
	HIGH	16.3	3.1	aA	22.5	5.2	bA	0	[0,0]	bA	2.88	0.05	b	3.17	0.21	a	45.5	a	48.9	a
Vision	CTR	13.8	2.4	aAB	5.0	2.0	aA	0	[0,0]	aA	3.17	0.06	a	3.08	0.08	a	44.8	a	46.9	a
	LOW	13.8	5.5	aA	15.0	4.1	aA	0	[0,0]	aA	2.97	0.12	ab	2.76	0.11	a	43.6	a	47.4	a
	HIGH	27.5	4.8	aA	30.0	4.6	bA	0	[0,1]	bA	2.74	0.09	b	3.13	0.18	a	44.3	a	47.6	a
Harper	CTR	25.0	6.1	aB	7.5	4.3	aA	0	[0,0]	aA	3.30	0.04	a	3.73	0.10	a	45.6	a	49.3	a
	LOW	47.5	3.2	bB	48.8	6.6	bB	0	[0,7]	bB	3.14	0.12	a	3.82	0.16	a	45.7	a	48.7	a
	HIGH	73.8	2.4	cB	60.0	4.1	bB	6	[0,9]	cB	2.80	0.05	b	3.62	0.09	a	45.5	a	49.2	a
Quartz	CTR	27.5	4.3	aB	11.3	4.7	aA	0	[0,1]	aA	3.40	0.09	a	3.38	0.09	a	46.2	a	48.8	a
	LOW	67.5	7.5	bB	55.0	9.8	bB	4	[0,8]	bC	2.81	0.13	b	3.42	0.03	a	45.5	ab	48.5	a
	HIGH	85.0	4.6	bB	93.8	2.4	cC	9	[3,10]	cC	2.23	0.12	c	3.06	0.15	a	44.5	b	48.5	a

^a First column shows average disease incidence, second column standard error and third column statistical differences performed with ANOVA ($P < 0.05$)

^b First column shows median disease severity, second column the interquartile range and the third column statistical differences performed with Wilcoxon rank-sum test ($P < 0.05$)

^c First column shows average yield standardised on 9% moisture content, second column standard error and the third column statistical differences performed with ANOVA ($P < 0.05$)

^d First column shows median oil content on dry matter basis and the second column statistical differences performed with a 5000 bootstrap replications of median differences ($P < 0.05$)

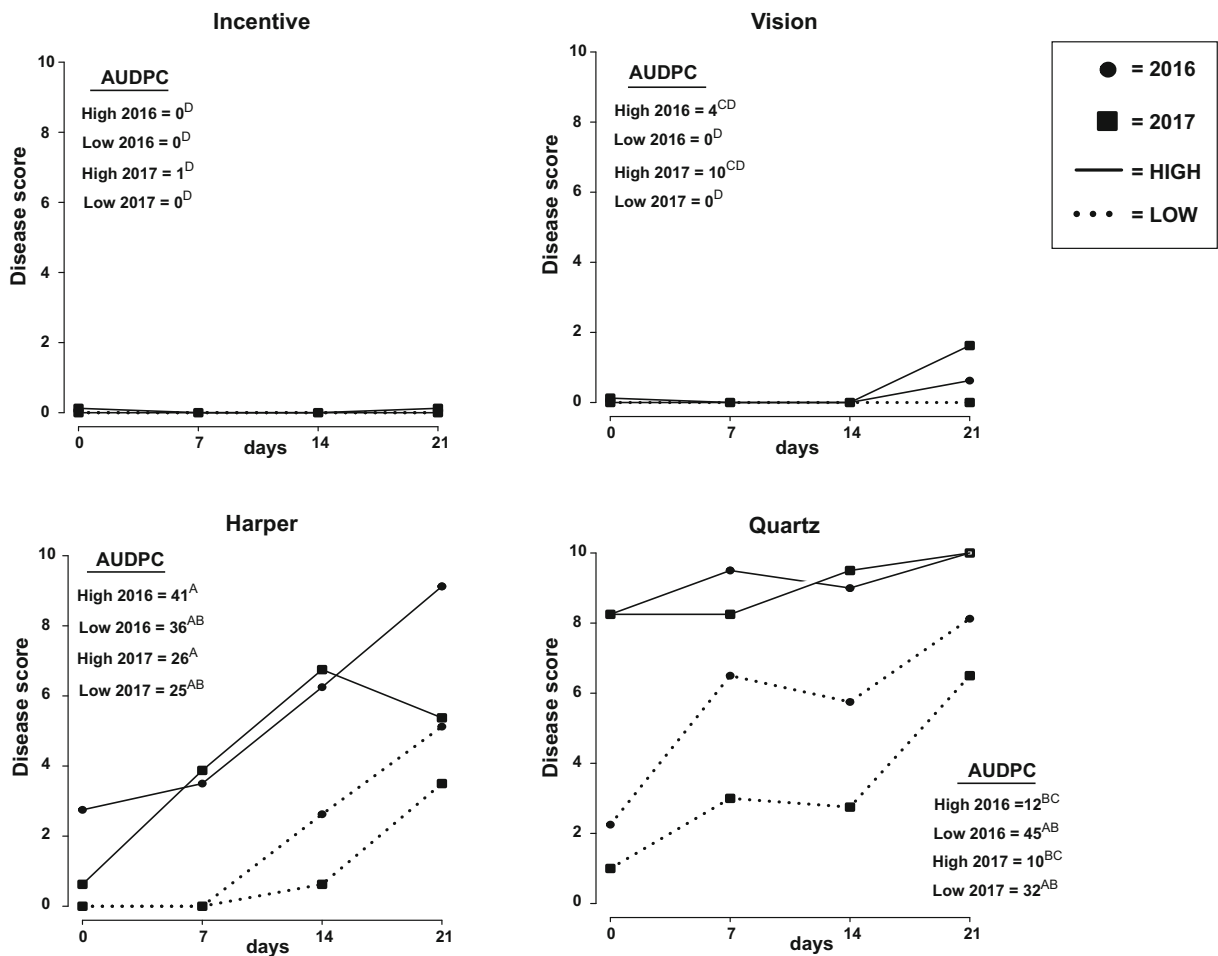


Fig. 1 Verticillium stem striping progression during the end of the cropping season. The Y-axis represents the median of the upper quartile plot disease score. The X-axis represents the number of days after the first scoring: 30th June and 27th June for

harvest years 2016 and 2017, respectively. The area under progress disease curve (AUPDC) was calculated and letters present significance levels for all panels collectively calculated with 50,000 bootstrap replications of median differences ($P < 0.05$)

(AUDPCs) for the lower and higher inoculum treatments were similar for both years (Fig. 1). Higher inoculum levels resulted in increasing incidence and symptom severity in Quartz for both cropping years (Table 1). Thus, in general, the symptom development of the four cultivars was consistent for the harvest years 2016 and 2017.

In order to evaluate whether the different symptom development between cultivars corresponded with economically important crop parameters, seed yield and oil content were measured. To this end, yield of oilseed rape were adjusted to 9% moisture content and oil content of the seeds was determined by Nuclear Magnetic Resonance (NMR) spectroscopy (Benchtop NMR Analyser – MQC, Oxford Instruments, UK). Differences in

oilseed rape cultivar and trial year impacted significantly on seed yield and oil content, whereas differences in the level of inoculum only impacted on yield and not oil content (Table 2). However, the impact of inoculum level was inconsistent between the two cropping years as significant differences in yield and oil content could only be found in the 2016 field trial (Table 1). In 2016, plots with the higher inoculum rate yielded significantly less than control plots for all tested oilseed rape cultivars. The highest yield impact was for Quartz that demonstrated a 34% reduction compared to the uninoculated control, which is within the range of previously made estimates of 10 to 50% (Table 1) (Dunker et al. 2008). Despite the presence of only mild disease symptoms, the yield in 2016 was also significantly

Table 2 Analysis of variance for effects on seed yield and oil content. Multivariate ANOVA was used: Yield/Oil content ~ Cultivar + Year + Inoculum level + Repeat

	Yield					Oil content				
	Df	Sum Sq	Mean Sq	F-value	P-value	Df	Sum Sq	Mean Sq	F-value	P-value
Main effects										
A: Cultivar	3	2.53	0.84	10.19	8.28×10^{-6}	3	33.81	11.27	16.21	1.95×10^{-8}
B: Year	1	2.93	2.93	35.39	5.66×10^{-8}	1	253.27	253.27	364.32	$< 2 \times 10^{-16}$
C: Inoculum level	2	2.14	1.07	12.94	1.22×10^{-5}	2	1.19	0.60	0.86	0.43
D: Repeat	3	0.38	0.13	1.55	0.21	3	9.59	3.19	4.58	5.02×10^{-3}
Residuals	86	7.12	0.08			86	59.79	0.70		

lower for Incentive (−11%) and Vision (−14%). The degree of yield reduction was similar for Harper (−15%), despite the observation that disease incidence and severity are significantly higher on this cultivar (Table 1). This indicates that stem striping is a poor indicator for predicting yield losses by *V. longisporum*.

Yield reductions for plots inoculated with the lower amount of inoculum were only significant for Quartz (−17%) while the other cultivars did not suffer from measurable yield losses (Table 1). Across all cultivars, the median disease symptom severity negatively correlated to yield (Spearman's $\rho = -0.53$, $P = 0.0001$). Similarly, the average disease incidence also correlated negatively with yield (Pearson's $r = -0.57$, $P = 0$).

In contrast to the observations in 2016, the relationship between Verticillium stem striping symptoms and yield was different in the 2017 trial, since no significant differences between the different inoculum treatments were observed in yield for any of the cultivars assessed (Table 1). Verticillium stem striping symptoms, incidence (Pearson's $r = 0.05$, $P = 0.73$) and severity (Spearman's $\rho = 0.09$, $P = 0.52$) did not correlate with yield. Thus, although these data comprise only two cropping years, this indicates that the impact of *V. longisporum* is inconsistent despite the occurrence of abundant disease symptoms. This might be due to differences in host colonization patterns of *V. longisporum* between the two years (Siebold and von Tiedemann 2013). Different weather conditions between the two years may have an effect on yield reduction by *V. longisporum*. Elevated temperatures can enhance the *V. longisporum* colonization on oilseed rape, however, this effect is not consistently observed (Siebold and von Tiedemann 2013). Watering

regimes were previously shown not to impact *V. longisporum* disease levels if grown under controlled greenhouse conditions (Lopisso et al. 2017).

The significant yield impact in harvest year 2016 showed that *V. longisporum* pathogen can be of economic significance in oilseed rape production. However, our inability to reproduce these yield reductions and the absence of significant oilseed rape yield reductions in a previous study indicate that significant yield reductions upon *V. longisporum* field inoculation only sporadically occur. As this study only comprises data from two consecutive cropping years, more field studies are needed to have a general assessment of the economic importance of Verticillium stem striping. Furthermore, to assess the impact of Verticillium stem striping under commercial conditions more accurately, propagule levels that are representative for *V. longisporum* infested agronomic soils should be used in future field trials, as we do not know if the inoculum amount applied on the soil in our study resembles the amount of propagules in commercially cultivated soils. To this end, species-specific primers can be used for *V. longisporum* DNA quantification in the soil (Debode et al. 2011; Tzelepis et al. 2017). In conclusion, the collection of yield data instead of striping symptoms in field trials would lead to a more genuine assessment of Verticillium stem striping resistance in oilseed rape cultivars, as we observed that striping symptoms can be untruthful predictors of final yielding outcomes.

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Compliance with ethical standards The authors declare that the research complies with ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

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