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EFFECT OF BRASSICA CROPS ON STEM BASE DISEASE OF WINTER WHEAT

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EFFECT OF BRASSICA CROPS ON STEM BASE DISEASE OF WINTER WHEAT

by

M NUTTALL[1], A S TURNER[2], R M GASKIN[1],
M SCRANCHER[2] AND P NICHOLSON[2]

[1] Morley Research Centre, Wymondham, Norfolk NR18 9DB

[2] John Innes Centre, Colney, Norwich, Norfolk NR4 7UH

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SUMMARY

Plots of winter wheat were established following a range of brassica crops on a sandy loam soil. There were significant effects of brassica type on the levels of brown foot rot on the stem base assessed by eye in March. Wheat grown after a fodder rape and a breeding line of oilseed rape appeared to have lower disease levels than other brassica crops tested. These differences, however, were not correlated with the levels of phenyl glucosinolates in the roots of the preceding brassica crop. Visual assessments at other dates did not give significant differences. Four pathogens were detected in samples throughout the season by PCR. These were *Tapesia acuformis, Rhizoctonia cerealis, Microdochium nivale* var. *nivale* and *M. nivale* var. *majus*. No significant differences in the amount of these pathogens were detected among the different brassica treatments. Yields were not significantly affected by preceding brassica type.

INTRODUCTION

Various reports of higher yields of wheat following oilseed rape in a rotation as compared to wheat following other break crops have been attributed to reductions in soil-borne pests and diseases. Under UK conditions it has been assumed that this phenomenon was, in part, a result of the exhaustion of eyespot debris on the soil surface following minimum cultivations. Other studies have suggested that this may be due to the toxic effects on nematodes and cereal root pathogens of phenylethyl isothiocyanate which is a result of the microbial breakdown of phenylethyl glucosinolate released by the roots of oilseed rape.

Significant differences in the levels of phenylethyl glucosinolate in the roots of different oilseed rape varieties and breeding lines identified in studies at the John Innes Centre may enable growers to enhance these yield effects by the selection of varieties producing high levels of this glucosinolate. Simultaneously, techniques have been developed at the John Innes Centre using DNA diagnostics which enable the accurate identification and quantification of the major cereal stem base disease pathogens in extracts from infected plants. These techniques could be exploited to assess the relative contribution of control of each pathogen to the yield effects from oilseed rape.

It was the objective of this experiment to determine the effect of brassica crops with differing levels of glucosinolates in their roots on stem base disease development, using visual and DNA diagnostic assessments, and yield of a subsequent wheat crop.

MATERIALS AND METHODS

Experimental design

A range of brassica crops was established in the autumn of 1995 on plots measuring 30.5 m x 24 m on a sandy loam soil at Newfound Farm, Colney, Norfolk. Six different cultivars or breeding lines were drilled at normal commercial seedrates on 11 September 1995 (Table 1).

Table 1. Details of brassica crops grown in 1995-96 before wheat

Treatment	Type	Cultivar/line	Level of phenylethyl glucosinolate
1	Winter oilseed rape	Apache	High
2	Winter oilseed rape	Broad leaf Essex	High
3	Winter oilseed rape	Breeding line JIC-1	Intermediate
4	Winter turnip rape	Debut	Low
5	Fodder rape	Hobson	Intermediate
6	Winter oilseed rape	Breeding line JIC-2	Low

The plots were arranged in a 6 x 6 latin square layout. The trial site received Butisan S (metazachlor, 500g/l) at 0.75 l/ha and Laser (cycloxydim, 200g/l) at 0.75 l/ha applied overall on 3 October for weed control and 40 kg/ha nitrogen applied overall on 8 October. Normal crop growth was allowed until 1 July 1996 when all seed pods, flowers and foliage were chopped by flail mower and the plant remains were deposited on the soil surface. The whole area was ploughed and pressed on 20 August.

Wheat, variety Riband, was drilled uniformly across all plots on 11 September, and subsequently all management was as normal for a farm crop, with fertilizer, herbicides, insecticides, fungicides and growth regulators also applied uniformly across all plots. In addition, two adjacent areas around the trial were drilled with winter rye, variety Amando, on 24 September. One had been cropped with Apache oilseed rape in 1995/6 (rye after brassica; RAB) and the other had been cropped with rye (variety Amando) (rye after rye; RAR).

The density of the wheat crop was assessed by plant counts on all plots on 25 October 1996. Stem base disease was assessed at intervals by visual examination and PCR-based assay of plants randomly sampled from each plot. Representative strips from each plot were harvested by combine on 6 August for yield and grain quality determinations. Similar assessments were also carried out on observation plots on the adjacent rye.

Table 2. Sampling dates

Sample	Date	Growth stage*	
		Wheat	Rye
1	17-20 December 1996	22-24	23-26
2	4 February 1997	22-29	23-26
3	19 March	25-29	23-29
4	13 May	37	55-59
5	2 July	77	81

*Tottman (1987)

Tissue preparation

Following visual disease assessment plants were passed to the John Innes Centre for DNA analyses. Early in the season a sample generally comprised 30 complete plants but later samples took the form of 30 tillers. Roots were removed as close as possible to the crown taking care not to lose any tissue from the stem base region. The size of the section of stem base used at each sample time tended to increase through the season as the region with lesions present increased. After removal of the roots the stem base was cut to the desired length and the upper part of the plant was discarded. The stem base sections were then chopped coarsely (with a razor blade) and transferred to pre-weighed flat-ended tubes. The tube weight and code for each sample were recorded. Tubes were placed, open, in a freeze drier for a minimum of 48 h depending on sample numbers in each batch and degree of wetness. After freeze-drying tubes were removed and closed. Freeze-drying allowed samples to be stored without risk of degradation of the sample prior to milling and DNA extraction. All tubes were weighed again to allow the dried weight of plant material to be calculated.

Three stainless steel ball bearings were added to each tube. The size of the balls varied throughout the season depending on the age, and hence hardness, of the tissue. The material was milled to a fine powder by processing in a ball mill (Glen Creston) for 5-10 min depending upon the age of the tissue. The milled material was transferred to 50 ml disposable centrifuge tubes for DNA extraction.

DNA Extraction

CTAB buffer (Nicholson & Parry, 1996) (30 ml) was added to a 50 ml centrifuge tube and the milled plant sample was added. Tubes were shaken well and incubated at 65°C for 1-2 h with shaking, at intervals, during incubation. Following incubation, 10 ml of 5 M potassium acetate and 5 ml $CHCl_3$ was added to each sample. Tubes were mixed by inversion, placed at -20°C for 30 min, and centrifuged in a large benchtop centrifuge at 3,000 rpm for 15 min. A standard volume (600 µl) of supernatant was removed from the upper (aqueous) phase to enable quantification of fungal DNA content on the basis of units per mg dry weight of plant tissue. This was added to a fresh tube (2 ml) containing 1200 µl of ethanol. A second sample was also removed at this time and treated in the same way to act as a 'backup'. The second tube was stored at 4°C. Tubes were shaken and left standing at 4°C for 1 h (or overnight). Tubes were centrifuged in a benchtop microfuge for 10 min and the supernatant carefully decanted off. The pellet was washed in 1 ml of ice-cold 70% ethanol and centrifuged at half speed for 10 min. The 70% ethanol wash was repeated and samples left to air dry.

The DNA pellet was redissolved in T.E. buffer at a rate of 0.1 µl/mg dry weight of plant material. DNA was quantified by SybrGreen fluorescence (see below) and concentration figures used to prepare sub-samples at fixed concentrations (typically 40 ng/ µl). DNA samples were stored at 4°C until use (or frozen for long term storage).

SybrGreen DNA quantification

Quantification was carried out according to the method of Hopwood *et al.* (1997). Standard dilutions of DNA (*Hind* III cut λ DNA) were prepared in a working 1:10000 dilution of stock

SybrGreen solution (Flowgen). The dilution series ranged from 0 - 2.0 ng/µl in 0.2 ng increments. Aliquots (100µl) of each dilution were pipetted into a microtitre plate and placed in a Titertec Fluoroscan II fluorescence plate reader. Emission was read at 538 nm after excitation at 485 nm. A standard curve relating DNA concentrations to excitation/emission figures was prepared (r^2 typically 0.99) and applied to excitation/emission figures from 1µl of each DNA sample in 100µl of working dilution SybrGreen. Where readings exceeded the range of the standard curve, appropriate sample dilutions were prepared and assayed. Duplicate readings were taken for each sample and mean concentrations determined. All samples were then diluted to a fixed concentration (typically 40 ng/µl) on the basis of the above quantification prior to PCR.

PCR amplification

Amplification reactions were carried out in volumes of 50µl containing 200 ng DNA as described by Nicholson & Parry (1996). Specific PCR was performed using the reaction components described by Nicholson & Parry (1996) on DNA extracted from infected plant material. The concentration of all DNA samples was adjusted to 40 ng/µl prior to PCR. Samples were applied to a preheated PCR block and denatured at 95°C for 2 min prior to commencing cycling. DNA was amplified using 'touchdown' PCR (Don *et al.*, 1991) to ensure specificity of product amplification. In this process the annealing temperature was 66°C for the first 5 cycles, and 64°C for the next 5 cycles. For amplification of DNA from plant material this was followed by twenty five cycles at 62°C. The temperature cycle used consisted of denaturation (95°C) for 30 sec, annealing (as described above) for 20 sec and extension (72°C) for 45 sec with maximal ramping rates between temperatures. A final extension step of 5 min was incorporated followed by cooling to 10°C until recovery of samples.

Quantification of fungal content using competitive PCR

Competitor fragments were generated for all the target fungi from the sequence of the 23 kDa extrisic polypeptide of photosystem II (Wales *et al.*, 1989) gene of *Pisum sativum* as described by Nicholson *et al.* (1997). DNA templates, for use as competitor molecules, were developed using the general method described by Förster (1994) in which two rounds of PCR were used to generate a competitor fragment which had 5' and 3' termini identical to the fungal 'target' primer sites but which had no internal sequence homology to the 'target' sequence. The process was carried out for all nine of the stem base pathogens to produce competitors for each. The competitor fragments were cloned into pGEM-T (Promega) and transformed into electro-competent *E. coli* (strain JS5) according to the supplier's instructions (Bio-Rad). Plasmids containing the competitor DNA fragment were harvested and purified using 'Wizard miniprep' system (Promega) according to the manufacturer's instructions. Stocks of each competitor DNA were diluted in TE buffer and stored at -20°C until use.

Since, if the proportion of competitor DNA template is too high it may saturate the reaction such that no fungal DNA could be detected, whereas, if the amount is too low, all plants would appear equally heavily infected, initial tests were carried out to determine the concentration of competitor DNA template for each primer pair that would result in approximately equal amplification of both fungal and competitor fragments when 0.1 ng of

fungal DNA was used in the PCR reaction (data not shown). Fungal total genomic DNA, in the range 1 pg to 10 ng, of the respective fungal species was then added to reagent mixtures containing the selected quantities of the relevant competitor DNA molecule prior to PCR. The reaction components and amplification conditions were the same as those for conventional specific PCR detailed above. Following amplification, the PCR products of each reaction were separated by electrophoresis through 2% agarose gel. Gels were stained with ethidium bromide, viewed under UV light on a 'Gel Doc 1000' system (Bio-Rad) and analysed using Molecular Analyst software (Bio-rad) to estimate the relative degree of amplification of the fungal and competitor PCR product in each sample. The relationship was then determined, for each dilution series, between the PCR product ratios and the amount of fungal DNA added to the reaction. This generated a standard curve, by reference to which the amount of fungal DNA, of the relevant species, in plant samples could be estimated.
Results were subjected to statistical analysis by analysis of variance (ANOVA) and by Tukey's Pairwise Comparison test.

RESULTS

Crop growth

There was no significant effect of brassica type on crop establishment. The mean plant density on the wheat plots was 163 plants/m². This compared with 218 plants/m² on the adjacent rye plots. Initial growth on the wheat plots was weak but uniform with no obvious disease present, but later development was normal on all plots. By contrast the rye grew well throughout its life.

Visual assessments

No disease was found on the material collected on 17-20 December sampling date.

Samples taken on 4 February showed low levels of brown foot rot (Table 3) but there was no effect of brassica type. There was no eyespot present at that date.

Eyespot was identified at low levels at 19 March (Table 3) but there were no significant treatment effects. However, brown foot rot infection levels at that date showed lower levels from Treatments 3 and 5 (Hobson fodder rape and Breeding line JIC-1). There were significant differences between Treatment 6 and Treatments 3 and 5, and also between Treatment 3 and Treatments 1 and 2.

Table 3. Stem base disease on 4 February and 19 March (% tillers affected)

Treatment	Eyespot (19 March)			Brown foot rot	
	Nil	Slight	Index	(4 Feb)	(19 March)
Apache	94.0	6.0	2.0	0.5	19.5
Essex	96.0	4.0	1.3	1.5	19.5
JIC-1	96.5	3.5	1.2	0.0	12.0
Debut	92.5	7.5	2.5	1.5	17.0
Hobson	96.5	3.5	1.2	0.5	14.5
JIC-2	94.0	6.0	2.0	0.5	22.5
LSD	NS	NS	NS	NS	6.39
Mean	94.9	5.1	1.7	0.8	17.5
RAB_1	100.0	0.0	0.0	0.5	2.0
RAR_2	98.5	1.5	2.0	0.0	3.5

1 Rye after brassica
2 Rye after rye

There were no significant treatment effects on observed stem-base disease at any of the subsequent sample dates (Tables 4 and 5). Assessments on samples taken on 13 May from adjacent areas of rye appeared to indicate higher levels of eyespot and brown foot rot on the rye grown after rye (RAR) than after rape (RAB). However, samples from 2 July appeared to show higher levels of eyespot and brown foot rot on the RAB compared with the RAR.

Table 4. Stem base disease on 13 May (tillers affected)

Treatment	Nil (%)	Eyespot Slight (%)	Eyespot Moderate (%)	Index	Sharp eyespot (%)	Brown foot rot (%)
Apache	56.5	43.0	0.5	14.7	1.5	92.0
Essex	51.5	48.0	0.5	16.3	0.0	94.0
JIC-1	50.0	49.5	0.5	16.8	0.5	90.5
Debut	57.7	42.3	0.0	14.1	0.5	87.1
Hobson	50.0	49.0	1.0	17.0	0.5	92.0
JIC-2	54.0	44.5	1.5	15.8	2.0	94.5
LSD	NS	NS	NS	NS	NS	NS
Mean	53.3	46.1	0.7	15.8	0.8	91.7
RAB	71.5	28.5	0.0	9.5	0.0	78.0
RAR	68.4	31.6	0.0	10.5	0.0	86.5

Table 5. Stem base disease on 2 July (tillers affected)

Treatment	Nil (%)	Eyespot Slight (%)	Eyespot Moderate (%)	Eyespot Severe (%)	Index	Sharp eyespot (%)	Brown foot rot (%)
Apache	37.5	51.5	9.5	1.5	25.0	1.0	74.5
Essex	26.7	57.3	14.1	2.0	30.5	2.0	76.4
JIC-1	36.0	49.5	13.5	1.0	26.5	0.5	77.5
Debut	37.0	43.5	17.5	2.0	28.2	1.5	74.5
Hobson	37.0	46.0	15.5	1.5	27.2	1.0	81.0
JIC-2	34.7	45.3	18.5	1.5	29.0	1.0	86.0
LSD	NS	NS	NS	NS	NS	NS	NS
Mean	34.8	48.8	14.8	1.6	27.7	1.2	78.3
RAB	34.5	48.5	16.5	0.5	27.7	0.0	75.0
RAR	37.5	53.0	9.0	0.5	24.2	1.5	67.5

DNA diagnosis

Samples were screened for nine pathogens at each sample time (*Tapesia yallundae* (W-type eyespot), *T. acuformis* (R-type eyespot), *Rhizoctonia cerealis* (sharp eyespot), *Microdochium nivale* var. *nivale*, *M. nivale* var. *majus*, *Fusarium culmorum, F. graminearum, F. avenaceum* and *F. poae* (brown foot rot). Only four of these species were detected on plants, *M. nivale* var. *nivale*, *M. nivale* var. *majus, R. cerealis* and *T. acuformis* (Table 6). *Microdochium nivale* var. *nivale* was the predominant pathogen detected within the trial and was present in all plots of all treatments at all sampling times. *Microdochium nivale* var. *majus* was detected at the time of the third (six plots) and fourth sampling (three plots) (19/3/97 and 13/5/97) but this pathogen was no longer detected by the time of the final sampling (2/7/97). *Microdochium nivale* var. *majus* was not detected in rye plants at any time. *Rhizoctonia cerealis* was detected at only the second sampling time when it was present in four plots (Table 6). Thereafter *R. cerealis* was, apparently, absent from all samples. *Tapesia acuformis* was detected in three plots, including two rye plots, at the time of the second sampling but was absent from all plots at the third and fourth sampling times. At the time of the final sampling (2/7/98) *T. acuformis* was detected in all plots (Table 6).

Table 6. Incidence of cereal pathogens at five timepoints.

	Sampling time (Pathogen)									
	20/12/96	4/2/97			19/3/97		13/5/97		2/7/97	
Treatment	Mnn	Mnn	Rc	Ta	Mnn	Mnm	Mnn	Mnm	Mnn	Ta
Apache	4	4	0	0	4	1	4	1	4	4
Essex	4	4	0	0	4	2	4	0	4	4
JIC-1	4	4	2	1	4	1	4	1	4	4
Debut	4	4	0	0	4	0	4	0	4	4
Hobson	4	4	0	0	4	1	4	2	4	4
JIC-2	4	4	1	0	4	1	4	1	4	4
RAB	n.d.	4	0	2	4	0	4	0	4	4
RAR	n.d.	4	1	0	4	0	4	0	4	4

Figures indicate the number of plot reps in which the pathogen was found (four plot reps per treatment).
Mnm : *Microdochium nivale var majus*
Mnn : *Microdochium nivale var nivale*
Rc : *Rhizoctonia cerealis*
Ta : *Tapesia acuformis*
n.d. : not determined
Absence of a column for a species at any given timepoint indicates failure to detect the pathogen.
In addition, *Fusarium avenaceum, F. culmorum, F. graminearum, F. poae* and *T. yallundae* were not detected at any timepoint.

Quantification of fungal pathogens

Only two pathogens were present in sufficient quantities to make quantification feasible. These were *M. nivale* var. *nivale* at all sample times (Table 7) and *T. acuformis* at the final sample time only (Table 8).

No significant differences were detected between the amount of *M. nivale* var. *nivale* in any of the brassica treatments. The amount of *M. nivale* var. *nivale* present in the 'rye after rye' appeared greater than that in the 'rye after brassica' at the penultimate and final sample times, however this effect was not significant.

At the final sample time the amount of *T. acuformis* in wheat following brassica line 'Debut' (intermediate glucosinolate) was significantly greater (P<0.05) than that in 'JIC1' (low glucosinolate). No differences were detected among other wheat treatments. The amount of *T. acuformis* was greater in 'rye after brassica' than in 'rye after rye' (P<0.05) at this sample time.

Table 7. *Microdochium nivale var nivale* fungal DNA load in plant tissue at five timepoints by competitive PCR.

	Sample time				
Treatment	20/12/96	4/2/97	19/3/97	13/5/97	2/7/97
Apache	0.81 (0.32)	0.67 (0.22)	0.25 (0.07)	1.62 (0.53)	1.28 (0.52)
Essex	1.00 (0.28)	0.61 (0.07)	0.34 (0.13)	1.92 (0.48)	1.36 (0.55)
JIC-1	1.11 (0.31)	0.62 (0.17)	0.27 (0.10)	1.09 (0.32)	1.48 (0.46)
Debut	1.06 (0.22)	0.71 (0.12)	0.37 (0.08)	1.51 (0.48)	1.18 (0.36)
Hobson	1.22 (0.39)	0.58 (0.10)	0.46 (0.22)	1.19 (0.36)	1.91 (0.64)
JIC-2	1.19 (0.57)	0.49 (0.04)	0.24 (0.07)	1.63 (0.29)	1.79 (0.88)
RAB	not sampled	0.90 (0.20)	0.15 (0.06)	9.78 (1.14)	16.26 (9.02)
RAR	not sampled	0.83 (0.22)	0.16 (0.03)	6.73 (1.39)	5.73 (2.34)

Figures are mean pg fungal DNA per ng total DNA extracted (3 PCR reps x 4 plot reps). Figures in brackets are standard errors of means.

Table 8. *Tapesia acuformis* fungal DNA load in plant tissue at the final sampling date (2/7/97) by competitive PCR.

Treatment	Fungal load
Apache	14.01 (10.01)
Essex	9.62 (5.59)
JIC-1	5.54 (4.68)
Debut	11.08 (4.21)
Hobson	30.42 (31.22)
JIC-2	9.07 (5.62)
RAB	93.77 (66.14)
RAR	7.50 (5.23)

Figures are mean pg fungal DNA per ng total DNA extracted (3 PCR reps x 4 plot reps). Figures in brackets are standard errors of means.

Yield

Treatments 4 and 6, both brassicas with the lowest levels of phenylethyl isothiocyanate, appeared to give the lowest grain yields. However, statistical tests show that these were not significantly different to the other treatments in the main trial, neither were there were any significant differences in grain specific weight or thousand grain weight (Table 9). Also there was no discernible yield difference between the RAR and RAB treatments.

Table 9. Grain yield, specific weight and thousand grain weight (at 85% dm)

Treatment	Grain yield (t/ha)	Specific weight (kg/hl)	Thousand grain weight (g)
Apache	7.42	70.7	44.1
Essex	7.37	71.4	44.1
JIC-1	7.33	70.2	42.7
Debut	7.11	71.3	44.4
Hobson	7.36	71.5	44.8
JIC-2	6.48	71.4	45.0
LSD	NS	NS	NS
Mean	7.18	71.1	44.2
RAB	6.46	70.5	29.4
RAR	6.25	68.7	27.0

DISCUSSION AND CONCLUSIONS

Visual disease assessment carried out on 17-20 December indicated that all plants were clear of infection. The results of diagnostic PCR, however, revealed the presence of *M. nivale* var. *nivale* in all samples. Thus PCR appeared to be detecting this pathogen before disease symptoms became apparent. Visual disease assessment at 4 February only revealed the presence of brown foot rot and PCR indicated that this was due to *M. nivale* var. *nivale* which was detected in all samples. PCR also detected the presence of *T. acuformis* (R-type eyespot) and *R. cerealis* (sharp eyespot) in a number of samples but these diseases were not apparent from visual disease assessment. Thus, PCR also appeared to be able to detect the presence of these organisms before symptoms became apparent.

Results from later in the season, however, indicated that the situation with respect to PCR and visual assessment was somewhat more complicated. Eyespot was detected by visual assessment at both 19 March (slight only on 5% of samples) and 13 May (slight on 46% and moderate on 0.7% of samples). PCR failed to detect either *T. acuformis* or *T. yallundae* at either of these sample times. Given that PCR was able to detect *T. acuformis* prior to symptom development, it is unclear why PCR did not detect this organism at either of these times. It is probable that the amount of plant tissue at these times was greater than at the 4 February assessment while the amount of the pathogen had not increased to a similar extent and hence may have fallen below the detection threshold for PCR. It is also conceivable that other pathogens were responsible for the symptoms attributed to the eyespot organism(s).

Sharp eyespot was observed at 13 May (0.8% of samples) and 2 July (1.2% of samples) whereas PCR did not detect *R. cerealis* at either sample time. Again the cause of the difference is unclear. PCR detected *R. cerealis* only at the February sample time, when it was detected in four of 32 samples. In this instance it is probable that the symptoms attributed to sharp eyespot were due to the R-type eyespot organism (*T. acuformis*).

Overall, the results from visual assessment, PCR diagnosis and yield did not indicate any significant effect of the phenylethyl glucosinolate level in the roots of any of the previous brassica lines on a subsequent wheat crop. Comparison with the 'rye after rye' and 'rye after brassica' material is complicated by the non-random position of these plots. Any differences between treatments may be due to position effects rather than treatment. Given this, it was none the less interesting to note that level of DNA of *M. nivale* var. *nivale* was higher in rye than in wheat irrespective of the previous crop in both the penultimate and final samples. Thus, it appears that rye variety Amando may be more susceptible to this pathogen than wheat variety Riband. Visual disease assessment suggested that levels of eyespot and brown foot rot were higher in RAB than in RAR at the time of the final sampling. Competitive PCR supported this finding and indicated that the level of *T. acuformis* was significantly higher ($P<0.05$) in the RAB than in the RAR. The level of *M. nivale* var. *nivale* in RAB also appeared to be higher than that in RAR although the difference fell just short of statistical significance. However, these differences were not reflected in yield which did not differ between RAB and RAR.

Overall, this work has not identified differences in levels of stem base disease, whether assessed visually or by PCR, that might be related to the phenyl glucosinolate content of roots of a preceding brassica crop. In the current experiment, the site was prepared by ploughing and pressing. This may have affected the distribution on any glucosinolate or isothiocyanates within the upper soil levels. It would be of interest to repeat this experiment using direct drilling of the wheat crop to minimise disturbance of the soil and to include wheat following wheat control plots.

This work has, however, provided insights into relationships between visual and PCR disease assessment which are of relevance to other, ongoing studies of stem base disease, including one funded by HGCA (project 0050/01/96). The current project has also enabled refinement of assay techniques which are being incorporated into the ongoing research.

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