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Investigation of high levels of erucic acid in consignments of double-zero oilseed rape varieties

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1. Abstract

Rapid tests for erucic acid content in double-low (also called ‘double-zero and ‘00’) rapeseed have identified a small proportion of loads with levels well over the 5% legal limit in extracted oil. A new standard of 2% is likely to result in a significant increase in the number of loads that are either rejected or subject to price penalties. This project was commissioned to investigate the possible causes or sources of contamination associated with elevated levels of erucic acid.

A set of 50 samples, collected from commercial oilseed rape crops at harvest 2017, were acquired to provide the core of the study. These were used to compare the analytical accuracy of the new near infrared spectrophotometer scanning (NIRS) with that of the more traditional but slower testing by solvent extraction and gas chromatography (GC). The same samples were also inspected for the presence and influence of oil-bearing and potentially high erucic weed seed. On a subset of 12 samples, tests were conducted at the single seed level to establish whether elevated erucic acid levels resulted from a general drift upwards or from the presence of seeds with elevated levels due to cross-pollination from high erucic rape crops or volunteers. In a separate work package, DNA tests on leaf tissue from volunteer plants, growing in oilseed rape crops for harvest 2018, were conducted to detect the presence or absence of the genetic trait for high erucic acid in the seed. The results from these tests were then related to the erucic acid levels measured in the crop at harvest.

Modern NIRS equipment was found to give a good comparison with GC analysis overall (over an erucic acid range of 0–40%) but gave reduced levels of accuracy in the 0–5% and 0–2% ranges. Consequently, in a small proportion of cases, the rapid test could incorrectly identify loads as having exceeded threshold values. Within the samples inspected, weed seeds were not present at levels that could have resulted in significantly elevated levels of erucic acid. Testing of a small number of oil-bearing weed seeds, identified and removed from the samples, confirmed the high-erucic status of some of these species and this reinforced the importance of controlling them in rape crops. Single seed testing confirmed the stability of the low erucic acid trait. Within the samples investigated, however, clusters of individual seed values, in a range between 10 and 50% erucic acid, clearly indicated the influence of high- or elevated-erucic acid volunteers as the principal causes of elevated levels of erucic acid. DNA extractions on leaf tissue of volunteer plants confirmed the potential value of this as a diagnostic/predictive test of risk levels presented by volunteers in rape crops in individual fields.

Farmers should ensure that any seed – purchased or farm-saved – has been tested and shown not to pose an erucic acid risk. Best cultural practices to minimise volunteers should also be followed, where any background threat exists. Grain samples, from individual loads moved, should also be retained for reference in the event of disputed test results.

2. Introduction

Contaminating levels of erucic acid in double-low oilseed rape are causing concern and have resulted in some loads being rejected and growers penalised. This study was commissioned to investigate the problem and to contribute to any crop management strategies to minimise any economic threat.

The European legal limit for erucic acid content in rapeseed oil has been 5% but there have been moves to reduce this to 2%. Legislation for this was approved by the European Commission Standing Committee on Plants, Animals, Food and Feed on 8 February 2019 but no timescale for implementation has yet been announced. The Federation of Oils, Seeds and Fats Associations Ltd. contract (currently FOSFA 26a) limit for erucic acid in rapeseed has been 2% for many years. This is the contract that applies to loads delivered to the main crushers and, most importantly for growers, specifies the basis for the payment of oil premiums. Because of the lack of sufficiently sensitive testing equipment, a 2% tolerance above this contract level has been allowed and the crushers have been willing to take loads with up to 5% EA. In the 2015–2017 harvests, elevated levels of erucic acid (EA) were detected, firstly in the extracted oil and then, as the problem became apparent with more sensitive testing equipment, in the oil profiles of grain loads of designated double-low rapeseed, on arrival at grain stores and crushing plants. Although the proportion of loads exceeding the 5% threshold has been relatively small, in the order of 1.5% of the total, some loads were considerably above this limit. For some growers, the consequences were rejected loads or price penalties. There is concern that the introduction of a new 2% EA legal threshold will greatly add to these difficulties.

Historically, because of the lack of sufficiently sensitive equipment to perform rapid assays on whole rapeseed, monitoring EA levels was largely confined to testing the oil quality after extraction, using gas chromatography (GC). In recent years, improved sensitivity of near infrared spectrophotometers (NIRS), already widely used for measuring quality parameters in grain crops, has led to their rapid-test use for oilseed rape. This has led to a more routine and comprehensive testing of loads at intake and much greater awareness of local variations in erucic acid content of crops. Although under rapid development, these tests still lack the full accuracy of GC and there are reports of individual loads being rejected or penalised, using NIRS, which have subsequently met the standard, using GC. Evidence from NIAB laboratories is that some NIRS equipment, with oilseed rape calibrations, has been grossly inadequate for the purpose.

Oilseed rape (*Brassica napus*) has a number of breeding forms. The majority are intended for food use and are characterised by low levels of erucic acid in the fatty acid profiles of their oil. Within the variety testing process, erucic acid level is not used as a performance criteria for new varieties but a test is required to assign a category (low = <2.0%; high = >2.0%) for distinctness testing as part

of National Listing. This 2% cut-off aligns with the FOSFA contract for food use oilseed rape. In reality, levels are normally much lower than this, typically <0.1%. This type dominates the national crop area of 5-600,000ha/annum. They are generally referred to as double-low varieties because of the low erucic values in their oil and the low glucosinolate content of the meal that remains after crushing and oil extraction. For the purposes of this report they will be referred to as Low Erucic Acid Rape (LEAR) types. A small proportion of varieties, for industrial use, have high levels of erucic acid (~50%) and approximately 25,000ha are grown for industrial use annually. These are commonly referred to as HEAR varieties (High Erucic Acid Rape). As can be seen (Table 1), using the example of two varieties recently tested at NIAB, as part of the National List trials programme, the principle change in the development of LEAR varieties was the re-distribution of erucic acid into increased oleic and linoleic fractions. The two types cross pollinate readily and, because of the paternal dominance of the genetic trait for high erucic content, any double-low plants receiving pollen from HEAR plants will produce a proportion of their seeds with elevated erucic acid levels at harvest. It should be noted that erucic acid content of varieties is only routinely tested in the seed supplied by breeders at the beginning of their statutory trialling process. This is because, once sown in trials with other varieties, the erucic acid content of the seed at harvest is immediately subject to influence by incoming pollen from plants in neighbouring plots, which may have different fatty acid compositions. Consequently, the test would be unreliable. In fact, in LEAR varieties, the erucic acid levels are so consistently low, at or below the limit of detectability, that a single test on the seed received is considered sufficient to permanently classify new varieties.

Table 1. Fatty acid composition of example varieties of HEAR and LEAR types – expressed as percent of all fatty acids present in the oil

	Fatty acid													
Name	Lauric	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Arachidic	Eicosenoic	Behenic	Erucic	Tetra-cosanoic	Tetra-cosenoic
Chain length	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0	C24:1
Type														
High erucic	<0.1	<0.1	2.40	0.12	0.69	10.30	11.40	9.40	0.62	4.54	0.63	53.96	0.18	0.95
Low erucic	<0.1	<0.1	4.38	0.23	1.48	64.66	18.27	8.56	0.50	1.00	0.26	<0.1	0.11	0.12

Source NIAB

The dichotomy in the crop arose in the early 1970s because of health concerns associated with erucic acid in dietary vegetable oils. During that period, commercial varieties of oilseed rape would, typically, have 30–44% erucic acid in the fatty acid profiles of their oil composition. The rapid expansion of the crop, both in Canada and in Europe led to a considerable research effort into its utilisation. Laboratory experiments, with rats, identified erucic acid as a possible causative agent in the development of heart lesions (Hulan *et al.*, 1976). At the same time, breeding effort resulted in

a dramatic reduction of erucic acid levels in the oil, first in spring rape (Canola) types and quickly transferring into the winter crop, which allowed the EU to introduce a legal of 5% in the commercial oil, seeking to avoid any potential health problems. Nutrition studies continued into the 1980s, generally indicating low levels of risk, to the point where Kramer *et al.,* (1983) were able to describe 'no-observable-effect-limits' (NOEL) for intake levels by weaner pigs, above which myocardial lipidosis and loss of contractile force of heart muscle might occur, although these were well within current feeding limits. An erroneous scare, associated with erucic acid in rape oil, came in 1981 when hundreds of deaths and many more poisonings of people in Spain were linked to consumption of rapeseed oil (McMichael, 1981) and erucic acid was implicated as the causative agent (James, 1994). This view was discredited when samples of the oil subsequently showed that it was a mix of vegetable oils and animal fats, treated with aniline for industrial purposes (Tabeunca, 1981; Gollob, 1981). Since then, there have been numerous, at times contradictory studies, on laboratory animals, livestock and human health. The topic is thoroughly reviewed by the European Food Standards Agency (2016).

The genetic inheritance of the high/low erucic acid trait in *B. napus* is quite complex and must be understood in order to evaluate some of the risks to crop quality from cross-pollination between plants of different types, either between fields or in-field *via* volunteer plants. As an interspecific hybrid species, it draws on both the *B. rapa* and *B. oleracea* genomes for many of its characteristics. Erucic acid levels are considered to be determined by the presence or absence of dominant and recessive alleles, at two gene loci (Harvey and Downey, 1963), referred to as *Bn-FAE1.1* and *Bn-FAE1.2*. These act additively to give a range of erucic acid levels, between high erucic and low erucic, in heterozygous crosses (Jönsson, 1977). Some doubt over this control mechanism was expressed by Cullen *et al.,* (2008), who failed to find the *Bn-FAE1.1* gene to be an adequate source of variation to affect erucic acid content. Recent correspondence with the breeders of high erucic varieties (personal communication) has confirmed the importance of the original 2-gene control mechanism in their programmes. To illustrate the principle, Figure 1 represents the theoretical outcome of a homozygous, haploid high erucic pollen grain crossing to a homozygous, haploid, low erucic ovule in the maternal plant. Here, the dominant and recessive alleles of the *Bn-FAE1.1* and *Bn-FAE1.2* genes are represented by 'A' and 'a' and 'B' and 'b' respectively.

AB	**x**	**ab**	**=**	**AaBb**
High (~50%)		Low (<0.1%)		Intermediate (~25%)

Figure 1. Theoretical outcomes for crossing of homozygous high erucic plants and low erucic oilseed rape plants

The seed arising from this cross (F1) is diploid and, because the trait is paternally inherited, will have an intermediate value, typically around 25% erucic acid, assuming 50% for the high erucic plant and <0.1% for the low erucic. If inter-crossing proceeds to the next generation (F2), the number of outcomes increases (Table 2). This is a theoretical presentation and it is understood, from discussions with breeders, (*personal communication*) that variation, around these predicted levels, can result from the activity of minor genes (Havlickova *et al*., 2018). It is also important to bear in mind both that oilseed rape is highly self-pollinating (>80%) and that the majority of cross-pollination is likely to come from immediately adjacent plants, rather than drift from neighbouring fields or insect activity. As already described, the pre-LEAR varieties had lower erucic acid contents than the modern HEAR varieties, in the order of 30-40%. Volunteer plants from that era will have survived, regenerating in successive oilseed rape rotations, crossing with the sown field crop varieties and further adding to variation in erucic acid contamination levels.

Table 2. Theoretical outcomes for a mixed homozygous/heterozygous population of oilseed rape (amphidiploid) – allele combinations and erucic acid level (descriptions and percentage)

		Pollen (Haploid)			
		AB	Ab	aB	ab
Recipient plant ovule (haploid)	AB	AABB High (~50%)	AABb Intermediate-high (37.5%)	AaBB Intermediate-high (37.5%)	AaBb Intermediate (~25%)
	Ab	AA Bb Intermediate-high (~37%)	AAbb Intermediate (~25%)	AaBb Intermediate (~25%)	Aabb Intermediate-low (~12.5%)
	aB	AaBB Intermediate-high (~37.5%)	AaBa Intermediate (~25%)	aaBB Intermediate (~25%)	aaBa Intermediate-low (12.5%)
	ab	AaBa Intermediate (~25%)	Aabb Intermediate-low (~12.5%)	aaBa Intermediate-low (~12.5%	aabb Low (<0.1%)

Given this background, several causes of elevated erucic acid levels in low-erucic rape crops are evident. They include cross-pollination from adjacent high-erucic crops and from high erucic volunteers within the crop, contamination of the sown seed, and the presence of oil-bearing weed seeds. The accuracy of testing at intake also requires investigation and a number of opportunities for human error to lead to contamination have to be considered, throughout the seeding/harvest/ storage/transport chain.

Occurrences of HEAR volunteers in LEAR crops, including crops in fields with no record of previous HEAR cropping have previously been reported and discussed in a study of factors affecting cross pollination in oilseed rape growing under UK conditions (Squire et al, 2008). Low levels of HEAR volunteers were found to be widespread and some HEAR-type impurities were also found in LEAR seed lots.

The current study was commissioned in order to provide an understanding of the emerging problem and identify any management changes, throughout the supply chain, that might minimize the risk to crop quality. The structure of the project was to:

1. Acquire a diverse set of fifty rapeseed samples, from harvest 2017, to provide material for the study
2. Compare and contrast the accuracy of GC and NIRS testing for erucic acid
3. Assess the contribution of weed seeds to the overall erucic acid content in grain samples
4. Perform forensic examinations of erucic acid content at the single seed level, in grain samples
5. As a separate work package, the project was required to investigate the possible contribution of volunteer oilseed rape plants, growing within oilseed rape crops for the 2018 harvest, to erucic acid contamination of those crops.

3. Materials and methods

3.1. Acquisition of rapeseed samples

The project specification was for the investigation of 50 x 250g samples of rapeseed, with a range of erucic acid values above and below the statutory intake standard. On request, samples were supplied from a range of sources including a crusher, two distributors and a number of agronomists. It was hoped that all the samples would be arrive fully documented for seed source and variety but those received from the crusher and distributors came under anonymous codes and with no further identity information. Those collected from growers and submitted via their agronomists came with information on seed source (certified or farm-saved) and type (hybrid or conventional). A total of 90 samples were received and put through an initial erucic acid screening, after which 50 samples were selected as the core material for the project.

3.2. Erucic acid testing methodology

3.2.1 Gas chromatography (GC)

Assays were conducted by heptane extraction of oil from crushed seed samples and analysis in a Perkin Elmer Clarus 600 chromatograph, following the methods set out in BS 684 Section 2.34, Preparation of Methyl Esters of Fatty Acids and Section 2.35, Analysis by Gas Chromatography of Methyl Esters of Fatty Acids. The tests, on 5.0g samples, extracted with 25.0ml heptane, were

duplicated. For a subset of samples, 50 x single seed testing was conducted, to investigate within-sample variation. For these tests, 0.2ml heptane/seed was used for extraction and the assay was adapted to split-less injection into the GC column.

3.2.2 Near Infrared Spectroscopy (NIRS)

Two NIRS machines were used. The initial project specification was for the use of an Infratech 1241 but after early consultation with industry specialists, the Foss DA 1650 was used as the principal NIRS comparator for the GC test values.

3.2.3 Infratech 1241 NIRS

250g samples were scanned. Three tests per sample were performed.

3.2.4 FOSS DA 1650 NIRS

Samples (150g) were scanned in a glass-bottomed chamber using a development calibration (Version 4) for rapid testing. The work was carried out by Openfield Ltd. who are jointly developing the calibration. Tests were duplicated.

3.2.5 Sample testing

All 90 samples were tested for erucic acid levels using GC. The 50 samples selected for the full study were tested at NIAB using the Infratech 1241 NIRS and then by Openfield Ltd using the FOSS DA 1650 NIRS. The results of the three methods were compared.

3.3 Sample purity examination and fractionation

3.3.1 Twenty-five samples, expressing the maximum range of erucic acid values in GC tests, were selected for grain purity inspection by the Official Seed Testing Station (OSTS) staff. The bulked samples (200g) were subdivided into 100g portions, using a mechanical divider, to ensure thorough mixing.

3.3.2 From each sample, a 100g sub-sample was examined in its entirety and all seeds present, other than the crop species, were removed, identified and recorded by number and weight and the weight by percentage of the sample calculated. The 100g sample weight (approximately 25,000 seeds) was based on the ISTA recommendation for the species. The cleaned residues were returned to the GC laboratory and re-tested for their erucic acid content and comparison with the initial tests before cleaning.

3.3.3 The second 100g samples were retained for reference.

3.4. Forensic examination of single seed erucic acid content

3.4.1 Twelve samples were selected for analysis at the single seed level, testing 50 seeds per sample to investigate within-sample variation using the adapted GC test described at 3.2.1.

3.5. Investigation of field volunteers within the 2018 crop

3.5.1 Leaf sampling

The work focussed on a cluster of farms in Nottinghamshire, where elevated levels of erucic acid had been reported in crops for the 2016 harvest season, and at a farm in Cambridgeshire, with a known history of HEAR cropping. Five fields were selected for study. At each location, on inspection, the crop rows were observed to be sufficiently wide apart to allow volunteers, growing between the rows, to be identified, with a good level of certainty, as different from the sown crop. During March, before stem extension, leaf samples were collected. In each field, sampling was conducted at five points along each of four tramlines, giving 20 sampling points per field. At each sampling point, a single leaf was collected from each of ten volunteer plants and bagged together as a bulk for cold storage.

3.5.2 Leaf tissue analysis for the *FAE1.2* allele

From the bulk samples, tissue samples, comprising a leaf disc from each of the ten leaves per sampling point, were sampled and DNA was extracted, using a Qiagen DNeasy® 96 plant kit. The extracts were run through an Applied Biosystems Stepone Plus qPCR machine to detect the *FAE1.2*, high erucic trait, allele, using Taqman technology (Cullen *et al*., 2008). A calibration had been prepared, using seeded bulk samples, prepared with leaf discs taken from known varieties of HEAR and LEAR plants raised, in seed trays, for that purpose. This allowed an estimation of the number of leaves, per batch of ten, with the *FAE1.2* allele. Sub-sets of the batched leaves from each field, showing the presence of the HEAR trait was selected for re-testing, at the single leaf level, to validate the batch tests.

3.5.3 Crop testing for erucic acid

At harvest, growers were asked to send in samples of rapeseed from each of the five fields. To avoid any undue interference with harvest activities, no fixed protocol for sampling was set but multiple samples were received from all sites and 10 harvest samples were received from each of the worst affected sites. All samples were tested for erucic acid using the GC method.

4. Results

4.1 Sample classification

Of the 90 samples submitted, 16 were specified as being grown from farm saved seed of conventional, open-pollinated varieties, 11 were from certified seed of conventional varieties and six were from certified seed of hybrids. A further sample was from a mixed heap, from both hybrid and conventional varieties. Four of the hybrid samples were grown from Clearfield™ hybrids, with herbicide tolerance to specific imidazolinone herbicides. The remaining 54 samples were without any useful identity information.

4.2 Preliminary screening for erucic acid

All 90 samples were tested for erucic acid in the fatty acid profile, using the GC method and the results are presented in chart form (Figure 2) and tabulated (Appendix 1).

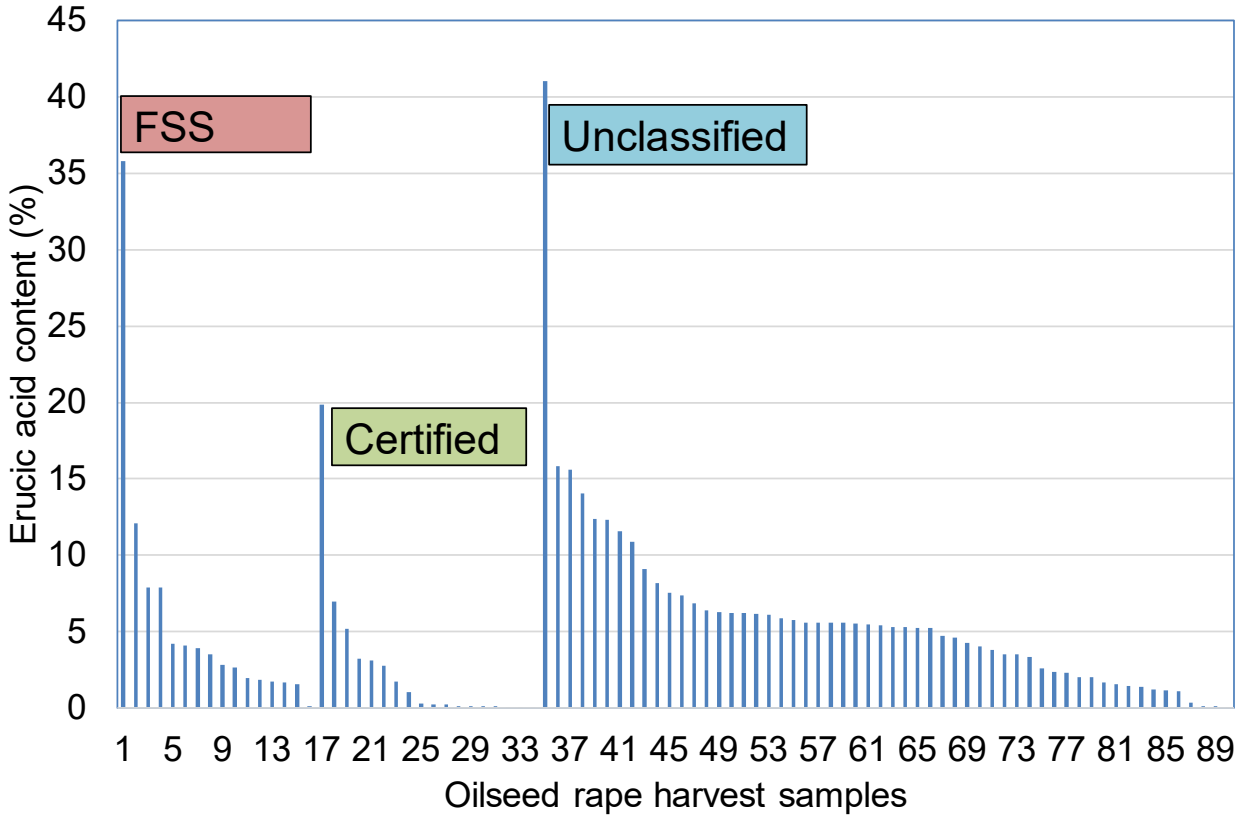

Figure 2. Erucic acid values of 90 crop samples of oilseed rape received, classified by seed origin (farm saved seed (FSS), certified, unclassified).

The high erucic extremes were 35.8% in the farm saved seed set, 19.9% in the certified seed set and 41.1% in the samples without classification. Removing these conspicuous outliers, the average values for these three groupings were 4.1%, 1.6% and 5.4% respectively. Table 3 provides a further breakdown, by type and erucic acid content, of the small number of samples from crops grown from certified seed, compared with the crops grown from farm saved seed. The overall sample size is clearly too small to provide a conclusive picture but the data are

indicative that further useful survey work that might be carried out to assess the risks of elevated erucic acid associated with different seed production mechanisms. Of the samples presented, a proportion arrived underweight and in selecting 50 samples for the full study, those with background information were prioritised while others were selected to provide a good range of elevated EA values.

Table 3. Erucic acid content of harvested crop, categorised by seed production type, for 15 samples of certified oilseed rape grown from different variety types and of 16 crops from farm-saved seed (% erucic acid)

	Farm saved seed	Farm saved seed excluding outlier	Certified conventional variety	Conventional varieties – excluding outlier	Mixed conventional and Clearfield hybrid	Certified Clearfield hybrids	Certified hybrids
Number	16	15	8	7	1	4	2
Mean	5.85	3.86	4.55	2.63	1.06	0.09	0.13
Maximum	35.80	12.08	19.88	6.96	-	0.12	0.14
Minimum	0.13	0.13	0.23	0.23	-	0.05	0.12

4.3 Comparison of solvent extraction/gas chromatography with whole seed/near infrared spectroscopy for erucic acid analysis

4.3.1 GC vs Infratech 1241 NIRS

For 50 samples, Figure 3 presents the comparison of erucic acid analyses from GC and NIRS using the Infratech 1241. Assuming the validity of the established solvent extraction and GC method, NIRS, using the Infratech 1241, provided a low level of accuracy, as evidenced by the R^2 value of 0.4347 and the relatively high proportion of points falling below the 5% line on the x axis (GC) but exceeding 5% on the y axis (NIRS). This confirms, as suggested by grain trading seed lab specialists, at the outset, that the Infratech 1241 and its calibration for oilseed rape, is unsuitable for erucic acid determination. In this set of samples, it tended to over-estimate the erucic acid content.

4.3.2 GC vs FOSS DA 1650 NIRS

For the same set of samples (as at 4.2.1) the FOSS DA 1650 NIRS gave a very close comparison with GC, with an R^2 value of 0.949 (Figure 4). Examining the points close to the 5% threshold value reveals a slightly poorer correlation (R^2 = 0.7738) but with only one out of 41 data points

exceeding the 5% level by NIRS testing which had fallen below 5% by GC (Figure 5). Taking this comparison down to the 2% level on the GC scale brought another reduction in accuracy, with an R^2 value of only 0.4979 and four out of the 27 data points exceeding 2% by NIRS which had fallen within that level on the GC scale.

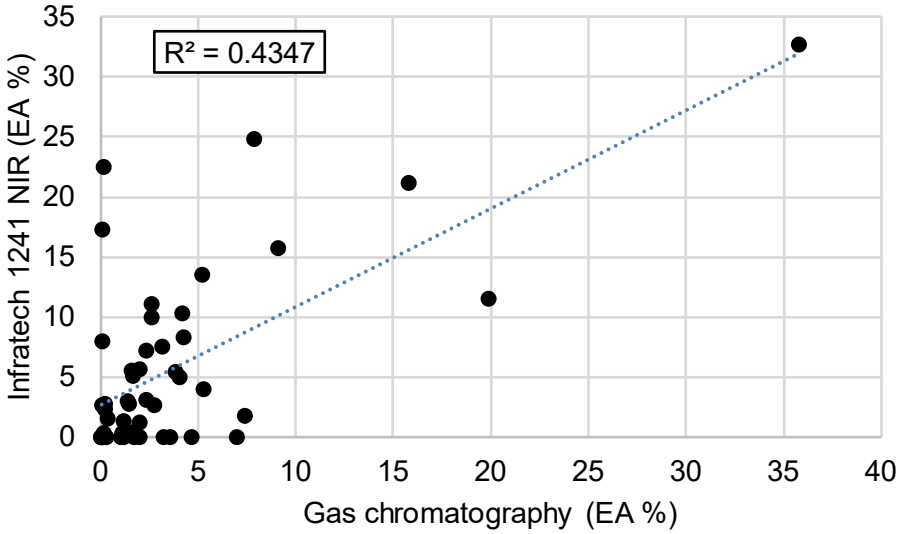

Figure 3. GC *vs*. Infratech 1241 NIRS erucic acid testing on 50 samples of oilseed rape (EA %))

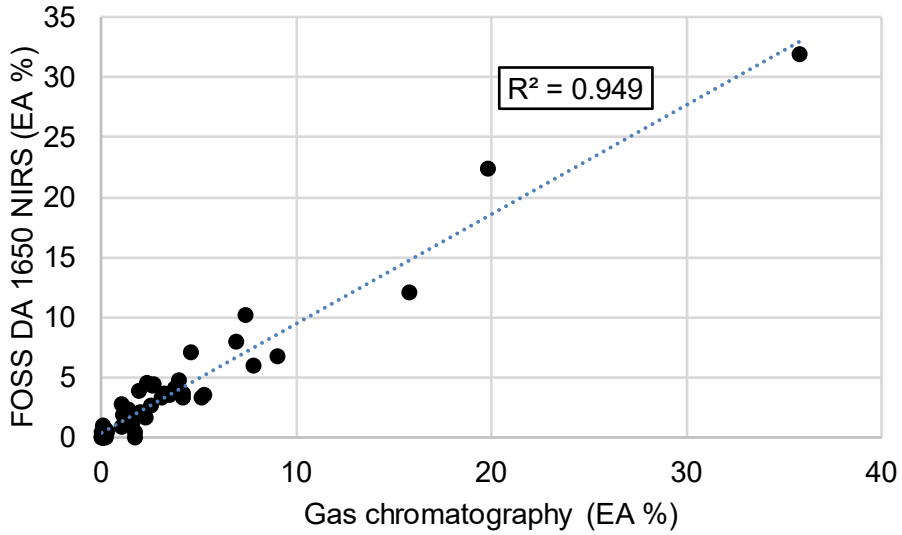

Figure 4. GC *vs*. FOSS DA 1650 NIRS testing on 50 samples of oilseed rape (EA %)

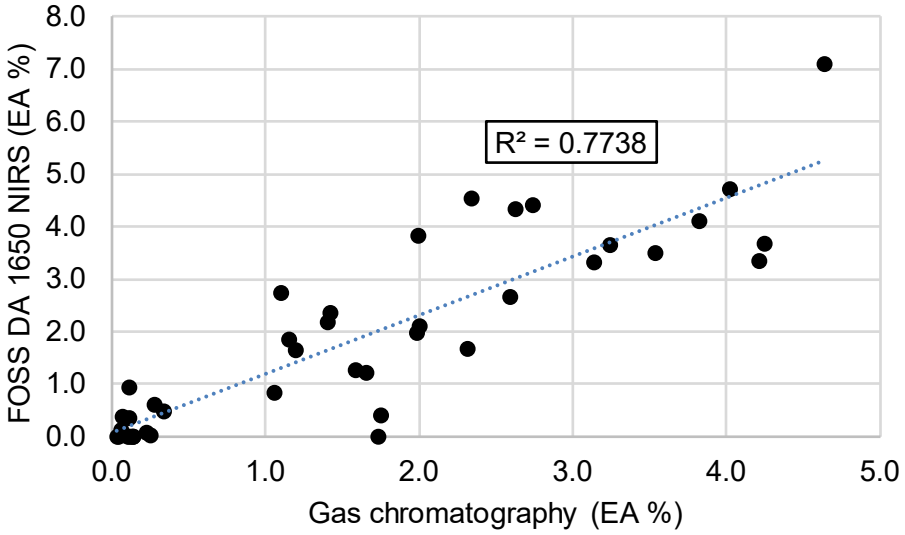

Figure 5. GC *vs*. FOSS DA 1650 NIRS testing up to the 5% erucic acid limit on the GC scale (EA %)

4.4 Sample purity the erucic acid influence of oil-bearing weed species

The 25 samples selected for investigation of weed admixture levels and their impact on the overall erucic acid content of the crop. The samples showed a good level of purity, with an average weed seed content of 0.31% by weight. Overall, seeds of 62 weed species were identified within the samples and these were counted and removed for weighing (Appendix 2).

In these samples, the impact of weed seeds on erucic acid levels was negligible, as indicated by Figure 6, which compares the erucic values of the 100g bulks, before and after seed cleaning. Here, the average change in erucic acid content, after cleaning was an increase of 0.04% but with 14 out of the 25 samples showing a marginal increase in erucic acid values after cleaning. These small margins of change are easily explained by variation within the oilseed rape grain sample itself. There was no evidence of weed seeds contributing to the three highly contaminated samples 14, 18 and 43.

The highest level of contamination was observed in Sample 32, where 1.37% was made up of non-crop species, principally hedge mustard (*Sisymbrium officinale*), which comprised 1.12% of the original sample. Table 4 summarises the incidence of oil-bearing weed seeds in the samples, their contribution by weight (%) and erucic acid values where there was an appropriate quantity to allow an oil analysis.

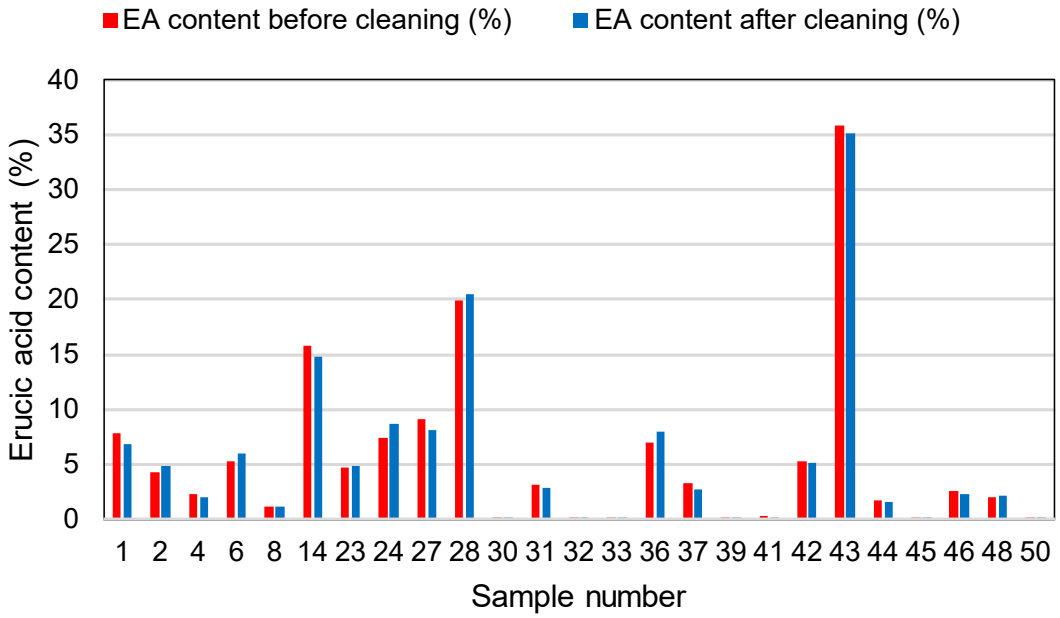

Figure 6. Erucic acid (EA) content of 25 samples of rapeseed, before and after cleaning to remove weed seeds

Table 4. Contribution of oil-bearing weed species and their erucic acid content (EA %) in 25 samples of oilseed rape (% by weight)

Weed species	EA	Sample number											
	(%)	1	2	4	6	8	14	23	24	27	28	30	31
Wintercress (*B. vulgaris*)	46.1*/24.5	0.06	0.01								0.09		
Charlock (S. arvensis)	42.5		0.01			0.06	0.01						0.04
Turnip rape (*B. rapa*)	41.1												
Black mustard (B. nigra)	37.0												
Hedge mustard (*S. officinale*)	23.9		0.01				0.01	0.01	0.01		0.01		0.02
Shepherds purse (*C. bursa-pastoris*)	0.36	0.01	0.01			0.01	0.01	0.01				0.01	0.01
Cleavers *(G. aparine)*	0.17	0.38	0.58	0.05		0.07		0.01	0.05		0.01		0.72
Total weed seed impurities		0.95	1.16	0.07	0.01	0.15	0.05	0.03	0.22	0.01	0.21	0.04	1.12

Weed species	Sample number												
	32	33	36	37	39	41	42	43	44	45	46	48	50
Bittercress (*B. vulgaris*)	0.01												
Charlock (S. arvensis)						0.01							
Turnip rape (*B. rapa*)												0.09	
Black mustard (B. nigra)						0.32							
Hedge mustard (*S. officinale*)	1.12	0.01				0.01	0.01	0.01			0.01		
Shepherds purse (*C. bursa-pastoris*)	0.13	0.01					0.01						
Cleavers *(G. aparine)*	0.01				0.01	0.06	0.01	0.01	0.01	0.10	0.81	0.01	0.01
Total weed seed impurities	1.37	0.02	0.00	0.34	0.01	0.52	0.10	0.02	0.03	0.35	0.94	0.10	0.01

4.5 Single seed investigations

Twelve of the cleaned samples from those described in Section 4.4 were selected for further detailed investigation, by oil extraction and analysis by gas chromatography, at the single seed level. The results are presented in chart form (Figure 7) and tabled (Appendix 8.3). In all twelve samples, contaminant seeds, with varying levels of erucic acid elevation, were identified. The individual seed values and their clustering were entirely in line with the predictive effects of a 2-gene, additive control mechanism, as suggested in the literature, as a result of heterozygous crossing, either in the field or in the seed crop. It was immediately evident that the erucic acid test values of the bulk samples resulted from the presence of contaminant seeds, with a range of elevated EA values, rather than a general drift in EA levels in the crop as a whole. Furthermore, the range and clustering of EA values in the contaminant seeds was closely aligned with the theoretical values suggested in Figure 2, although the 30% EA grouping appears to be the actual field value for the 'intermediate' cross rather than 25% and other clusters show a general 5% elevation above predicted levels.

Usefully, sample 48, illustrates the impact of the presence of a single full high erucic seed (50% EA) in a batch of fifty seeds of otherwise <0.1% EA values. The overall value is raised to 1% EA in the 50 seed batch. This compares with the cleaned source bulk sample value of 2.11% EA and provides a further illustration that, in the cases of low-level contamination, sampling will add its own degree of variation to the test result, with a high risk of missing contaminant seeds. A single, high EA seed is itself an unlikely occurrence as, if the seed was from a high erucic plant, growing in the crop, we would expect to see evidence of cross-pollination in the form of other seeds of intermediate EA values.

Sample 43 provides a complete contrast to Sample 48. Here only 10 seeds out of 50 can be classed as low EA. The clustering of values of the seeds with elevated EA conforms very well with the predicted values from Figure 2 and the almost continuum of values from 35% to 52% EA supports the concept of additional variation, brought in by minor genes, suggested in the introduction. This appears to have allowed overlap between the theoretical high and intermediate-high values postulated. This heavily contaminated sample was known to have been grown from farm saved seed of a conventional variety. Some of the samples (14, 23, 37) showed no representation at the 50% EA level, indicating the absence of modern full high erucic acid plants as contaminating occurrences in the those fields. This could reflect either inadequate sampling, or contamination arising indirectly from crops originally grown in the 1970s and perpetuated through periods of dormancy and successive rotations, with lower maximum EA values. One scenario missing from this set of samples, is a clear indication of cross-pollination from nearby HEAR crops, where incoming high erucic pollen, fertilising plants in LEAR field crop, would create a major cluster of intermediate values but no high values and no intermediate-high, or intermediate-low

values. Each set of 50 single seed tests, reported here, provides a ‘finger print’ of the history of oilseed rape cropping in the field it originated from. Testing more seeds would undoubtedly provide more robust evidence in each case but this was beyond the scope and budget of the project. It should be noticed that only Sample 42 came from a farm with a history of HEAR cropping and this was more than 10 years previously.

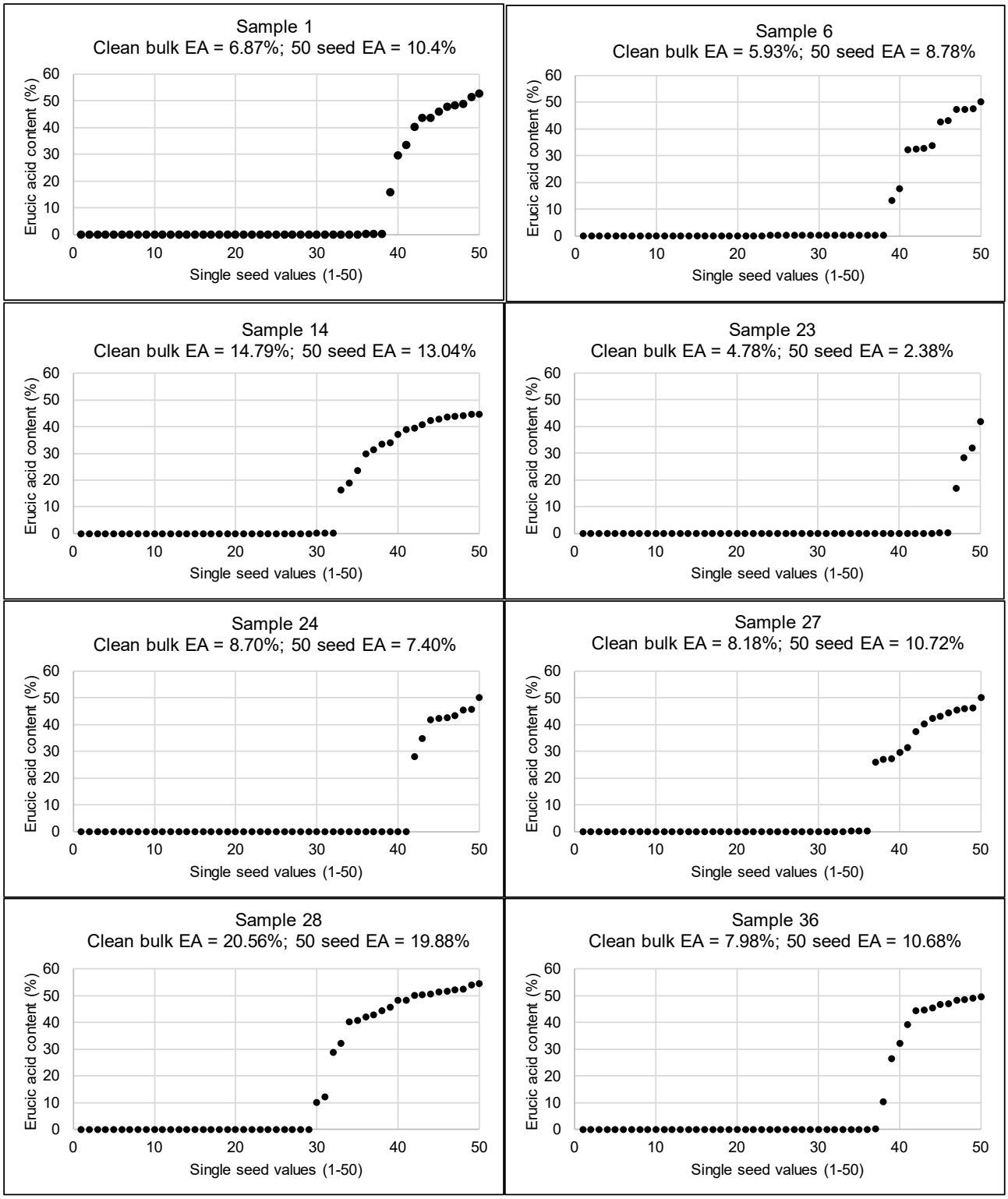

Figure 7. 50 x single-seed erucic acid analyses for sub-samples from 12 double-low oilseed rape crops exhibiting a range of elevated erucic acid content

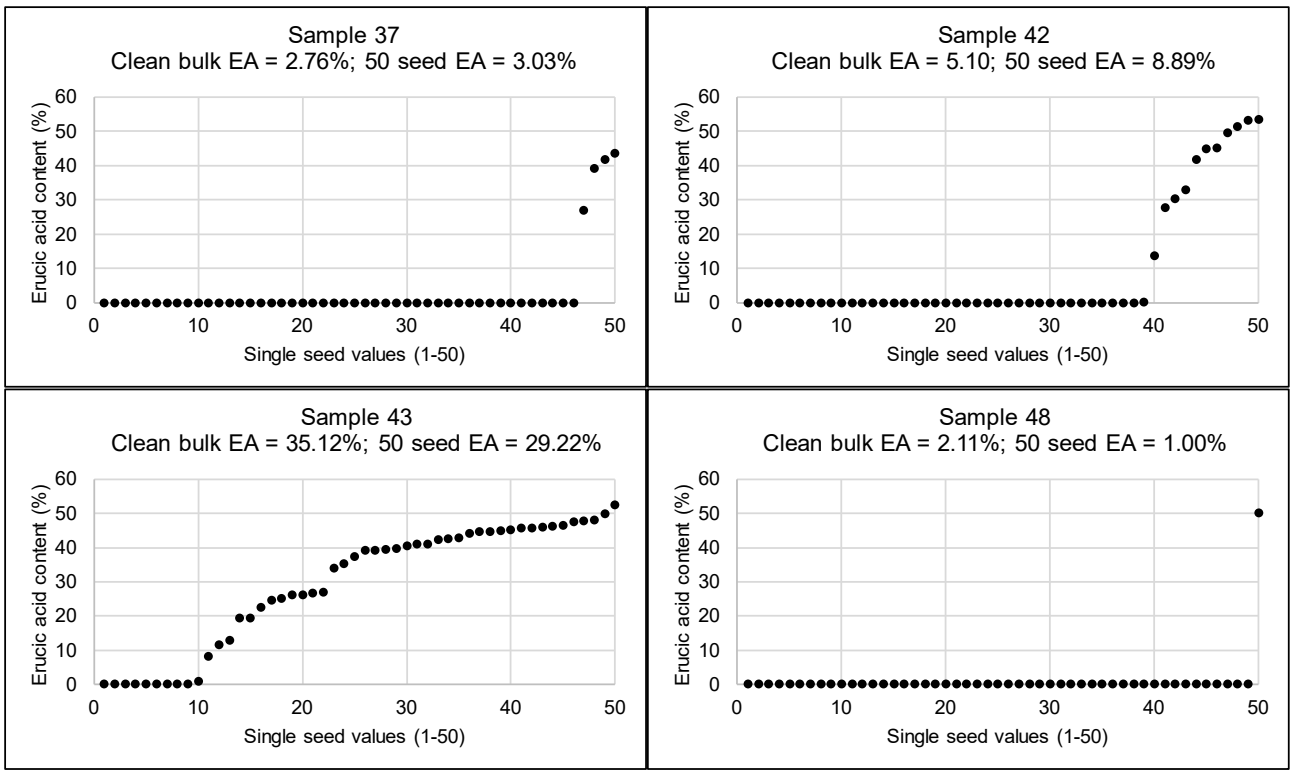

Figure 7. (Continued)

4.6 Investigations into erucic acid status of field volunteers in commercial crops

This part of the study was, essentially, a pilot investigation into the practicalities of examining the potential threat to crop quality from volunteer rape plants, growing in commercial crops. The five crops included certified conventional varieties (Fields 1 and 2) on a farm with a history of HEAR cropping and three crops of certified hybrid varieties on farms with a recent history of EA contamination but no history of HEAR cropping.

4.6.1 Calibration curve for the *FAE1.2* allele (dominant for high erucic acid production in the seed).

Figure 8 presents the cycle threshold (Ct) values obtained for DNA samples extracted from sets of 10 leaf samples, starting with a known low-erucic acid origin and progressively 'spiked' with 1-10 leaf samples of known high erucic acid origin. An increase in Ct value indicates a reduction in the quantity of target DNA present in the sample, thus requiring more PCR cycles to reach the detectable level (where HEAR DNA is present). The lower Ct values observed for the single HEAR leaf spike indicates that the *FAE 1.2* taqman assay did not perform efficiently when HEAR DNA was diluted with LEAR DNA (as reported by Cullen *et al*., 2008).

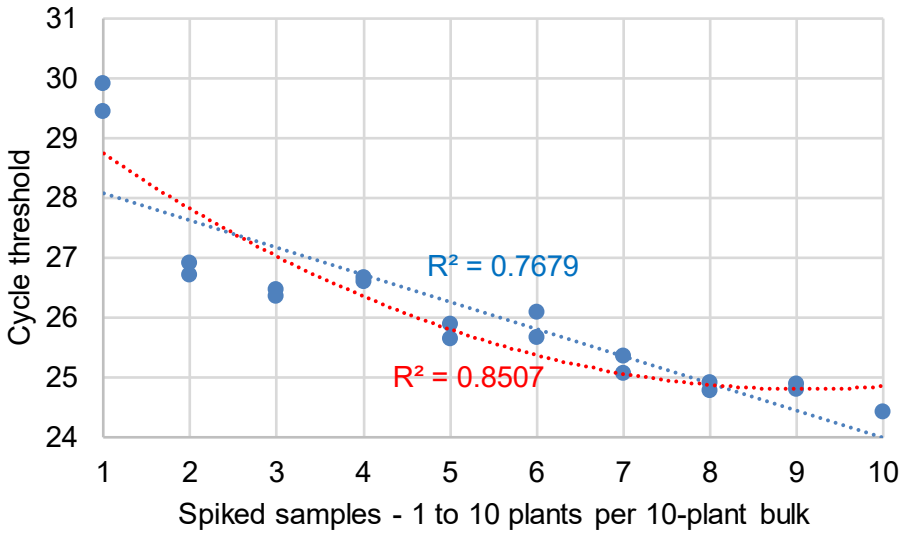

Figure 8. Graph displaying cycle threshold values for *FAE 1.2* in for DNA samples extracted from bulks of 10 low erucic acid rape leaf samples, progressively spiked with 1 to 10 HEAR leaf samples.

4.6.2 Analysis of bulked leaf samples from volunteer plants growing in five commercial oilseed rape crops

Acknowledging the lack of complete precision exhibited by the calibration curve, the leaf analyses were initially assigned to predictive groupings:

0 high erucic plants per 10 plant bulk
1-3 high erucic plants per 10 plant bulk
4-6 high erucic plants per 10 plant bulk
7-8 high erucic plants per 10 plant bulk
9-10 high erucic plants per 10 plant bulk

Using these groupings, the predicted erucic acid status of the volunteers in each commercial field, based on bulk samples, is given in Figure 9.

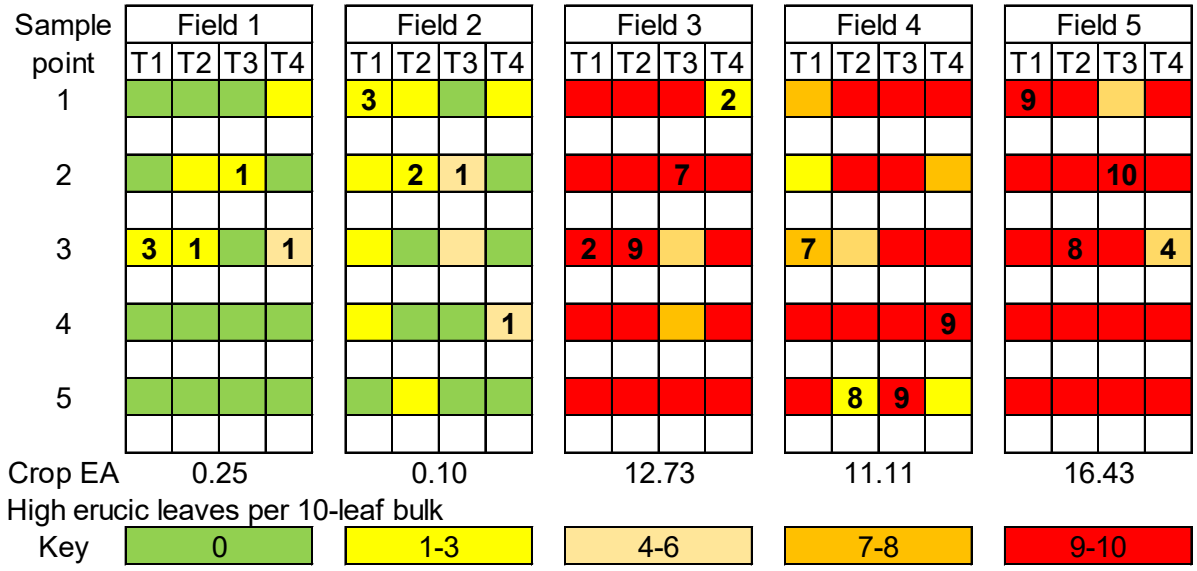

Figure 9. Summary of predicted erucic acid status in field volunteers based on bulks of 10 leaf samples, actual number of high EA leaves per bulk (where tested), and the EA status of the farm crops at harvest (Crop EA,%)

At the outset, the project team had anticipated low to medium levels of high erucic plants in the bulks and had undertaken to analyse bulks with indications of high erucic presence at the single leaf level. Because of the very high level of contamination in fields 3-5 this was not possible within the budget and staff time constraints and a subset of four bulk samples per field was selected for 10 x single leaf testing. The results of these, expressed as number of leaf disks with the *FAE1.2* allele are given. At harvest, the growers collected samples and sent these in for testing. The average crop erucic acid is given for each field (Crop EA) in Figure 9. These showed low levels of crop contamination in fields 1 and 2 but excessive levels of contamination in fields 3-5. Fields 1 and 2 were on land with a known history of HEAR cropping but only a small proportion of the

volunteers had the high erucic trait. The crops had been established with a Sumo drill/sub-soiler combination. Fields 3-5 had no record of HEAR cropping and were sown with certified hybrid seed targeting 40 plants/m^2.

Three of the growers with fields 3-5, suspecting a high level of EA contamination risk, sent in 10 harvest samples for each field and the individual test results are given in Figure 10. The variation in erucic acid levels within the samples from all three fields was reflective of the patchy nature, and variable density of the volunteers observed by the team collecting the leaf samples.

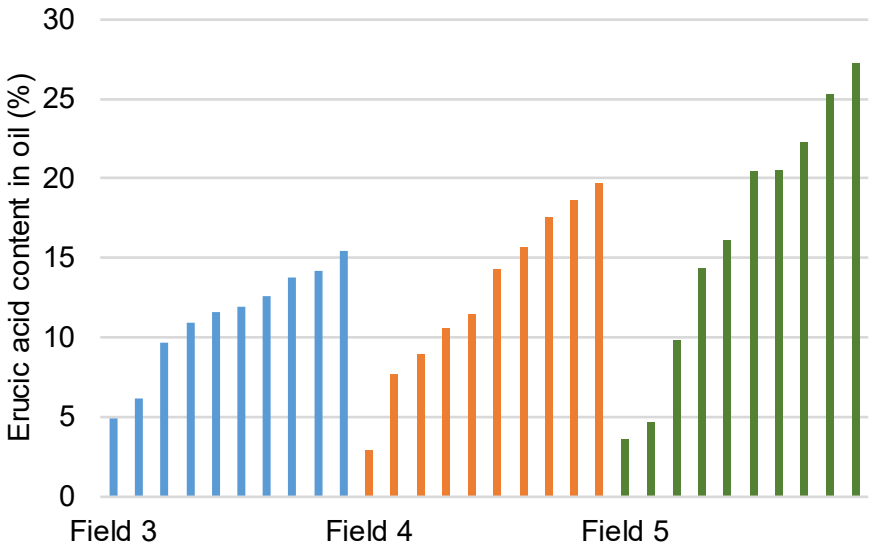

Figure 10. Erucic acid content in oilseed rape samples from three fields infested with oilseed rape volunteers arranged in ascending order of EA content

5. Discussion

While the proportion of loads failing to meet the erucic acid standard of 5% in the fatty acid profile has been relatively small, in the order of 1.5% in recent years, loads that do fail can be well in excess of this standard and present a major problem for the growers affected and end users. The introduction of a strictly applied 2% standard would be more problematic and could leave more growers penalised, or with unsaleable crops.

A common report, from growers with loads penalised for elevated EA, has been that, on re-test with GC, their EA levels have been shown to be satisfactory. The first element of the study has shown that modern NIRS equipment is much improved compared with older models and can give a very high level of correlation with GC testing, with an R^2 value in excess of 0.9, taken over the full range of sample values investigated in this project. In the critical 0-5% the correlation is less good (R^2 = 0.77) and in the 0-2% range there is considerable inaccuracy (R2 = 0.5). This means that there will be a likelihood of samples being over-estimated for their EA content. The commercial teams working on NIRS testing are insistent that their calibration of the equipment is continuing to improve and to deliver increased accuracy, adjusting for seed moisture content, in particular. It remains to be seen how the new 2% standard will be applied and whether or not a tolerance will be built into rapid testing at point of delivery. For the time being, it is in the interests of growers and grain traders to retain their own reference samples from each load that is transported, to allow retests in the event of disputed EA values.

The initial screening of the 90 samples submitted for the project showed an extremely high range of EA content, of from <0.1 to 41.1%. Crops grown from hybrids appear to carry the least danger of generating elevated EA levels. All hybrid seed will be certified. Crops grown from certified seed of conventional varieties carried more risk and those grown from farm-saved seed of conventional varieties, the greatest risk. The difference between crops grown from hybrids and conventional seed is likely to come down to the more exacting seed production methods for hybrids. Hybrid breeders will, typically, use greater isolation distances and seed lots are subject to isozyme tests for hybridity, which would show up the presence of off-types, including HEAR crosses. Nearly all hybrids are produced in southern Europe and very unlikely to encounter cross-pollination from other crops and particularly, high erucic crops. They also tend to use wide rows and direct drilling and separate male and female blocks. All of this makes the avoidance of off-type contamination easier than in conventional seed production. Certification of conventional varieties is also conducted within well-established regulations but low levels of contamination from volunteers or cross-pollination between crops can never be ruled out. A proposal from this report will be for consideration of adopting EA tests as standard for seed producers.

Erucic acid in the seed oil of weed species was not found to be a significant contributor to the elevated EA levels in the samples investigated. Nevertheless, contamination by brassica weeds cannot be excluded from the wider problem. Charlock, for example, with around 40% EA, is difficult to control, particularly in mild winter conditions, as the available herbicides are largely dependent on de-waxing the leaves and rendering the plants susceptible to frost kill. Uncontrolled charlock will flower and set seed at much the same time at the rape crop and the seed will contaminate the harvested grain. This may well have been a widespread factor in the 2016 and 2017 harvests, when the elevated EA values were being picked up by the new NIRS tests. Use of Clearfield® varieties and the associated imidazolinone herbicides now present the opportunity to control brassica weeds in the autumn and we understand that this is, in part, driving the increased uptake of these varieties, estimated at 14% of the oilseed rape area for 2019. They must be used as part of an integrated approach to manage weeds and volunteers throughout the crop rotation. As a generalisation, wild brassicas do not hybridise with oilseed rape in field conditions. An exception to this is a population of wild turnip rape in Yorkshire, which has been found to hybridise with oilseed rape and may have resulted in hybrid volunteers with elevated erucic acid content (Norris, 2002).

The single seed testing was highly indicative of the stable nature of the low erucic trait, with the majority of the seeds, in most samples, at the limit of detectability, at, or around <0.1% EA. We would expect any environmental sensitivity of the biosynthesis pathway to be characterised by a range of values just above the zero line and these were not present. The clustering of elevated values at points in the 10-55% range was strongly in line with the outcomes predicted from knowledge of the quantitative genetic regulation of the biosynthesis of erucic acid in oilseed rape. In the majority of cases, full high erucic rape levels were found in a small minority of seeds. These are most likely to arise from the presence of full HEAR plants in the crop. The clusters of intermediate EA values suggest that high erucic rape plants have been present in those fields and have cross-pollinated with the sown double-low crops over multiple rotations. If we accept this evidence then control of volunteers and avoidance of farm-saving on any land associated with reports of elevated EA levels must be prioritised. Volunteers can be minimised by three main approaches: direct drilling, to minimise soil disturbance; creating stale seedbeds and removal of volunteers before drilling the rape crop; growing Clearfield® and spraying off the volunteers once the crop is established. Of these, the stale seed bed approach is perhaps the least useful, when following wheat, as there is usually not enough time to cultivate and get the volunteers to emerge before the urgency to drill builds, especially with the current pressure from flea beetles.

It is almost impossible to speculate with any confidence, about the origins of high- or elevated-EA volunteers, on any one farm, unless there is well documented evidence of HEAR cropping that can be referred back to. As already described, some of the HEAR plants can have originated back in the 1970s and regenerated in successive rotations. The ‘industrial rape’ cropping as part of the

Arable Area Aid Scheme, in the 1990s to early 2000s, included an expansion of the HEAR crop which may not have gone as well recorded on farm as it is today. As described in the introduction, the earlier gene flow study (Squire *et al*, 2008) also found similarly inexplicable incidences of high erucic volunteers in fields with no recorded history of HEAR cropping. One source of high erucic volunteers and pollen can be forage rape, where older varieties are high erucic types. Forage rape crops, while utilised before flowering and seed set, are just as likely as oilseed rape to leave a proportion of seeds dormant in the ground at drilling, to emerge years later. Seed production fields of forage rape will inevitably leave a burden of seeds in the soil. A conspicuous example of this is the tall, pale flowered variety, Hobson, which is often seen emerging above rape crops at flowering.

Intermediate erucic acid volunteer plants might also have arisen from pollen flow from nearby HEAR crops or roadside volunteers, either wind-blown or carried by bees. Wind-blown pollen is usually not considered to pose a major source of cross-pollination as the pollen is regarded as too heavy to travel more than a few metres but remains a potential threat in dry, windy conditions at flowering. There is no regulation for HEAR/LEAR crop separation, although growers of HEAR normally adopt a 50m separation distance, by tradition, as a courtesy to neighbours, to minimise the threat. Pollination by bees and other insects can take place over distances of up to three kilometres and must always be considered as potential source of pollen flow. The majority of bee pollination, estimated at 10-15%, will be within a crop, rather than between crops, however. A MAFF project report (Ingram, 2000) estimated isolation distances, between crops, required to maintain given cross pollination levels below given percentages as: 1% - 1.5m; 0.1% - 10m, 0.1% - 100m. A further source of field contamination can be from seed transferred between HEAR and LEAR fields in combine harvesters that have not been cleaned out adequately between crops, and which is subsequently spilled.

As a guide to general risk levels, a summary of erucic acid levels in 915 commercial samples of oilseed rape tested by NIAB prior to sowing in 2018 (Appendix 4) has been included for information. The samples have been anonymised but will comprise a mixture of certified and farm-saved seed. Forty-four percent were assessed at <0.1% EA and 37% at 0.1-1.0%. Six percent were tested in the 1.0-2.0% EA range and would have presented a marginal risk for exceeding a 2% threshold if re-drilled into a field with an existing level of HEAR volunteers. The remaining 12% were well above the safe level for re-drilling.

The DNA investigation of volunteers in five commercial crops of oilseed rape was designed as a pilot study to assess the direct impact of volunteers on crop quality. It provided a good indicator of EA contamination levels in the harvested grain but needs further development to provide a fully effective predictive tool. Using the assay for the *cm-FAE1.2* allele adequately identified the

presence of the high erucic trait in single plants but lost accuracy when the assay was carried out on bulks of 10 leaf samples. The value of using this test is not so much for any benefit in the current season crop but more as a predictive test to apply to oilseed rape volunteers germinated from soil samples from future season fields. The test could have limited value in established crops, sown on wide rows and where there was access to precision inter-row herbicide spraying or cultivation but these options are few and reducing with the loss of key active ingredients. Using DNA testing to identify elevated EA volunteers in other fields within the rotation might have merit. This would involve growers conducting classic W shaped soil sampling exercises across fields and putting the samples into seed trays to germinate any rapeseed present. Where these emerged in large numbers, submitting leaf tissue to laboratories equipped for the DNA test would give a good indication as to the level of the high EA risk present. This, in turn, would give growers the opportunity to manage the risk by avoiding those fields for oilseed rape cropping, modifying their cultivations to minimise volunteer emergence, or to opt for Clearfield® cropping and the associated herbicides, to eliminate the volunteers.

AHDB (2018) has produced updated general guidelines for the avoidance of high erucic contamination of rape crops.

6. Conclusions

Within the scope of the project and of the 50 harvest samples investigated, and in the absence of any direct evidence of cross pollination from neighbouring HEAR crops, or contamination by brassica weed species, it is concluded that volunteer oilseed rape, with high, or elevated erucic acid levels, has been responsible for the high levels of erucic acid found in the samples. This view is strongly supported by the evidence from the single seed erucic acid tests on the 12 harvest samples from 2017 and the DNA testing of volunteer leaf tissue and subsequent grain testing at five locations in 2018. Over time and because of the genetically dominant nature of the high erucic trait, the problem is amplified and moves around the farm with the practice of farm saving conventional seed. Tight control of volunteers, through cultural practice and sowing only seed tested at a high level of purity, preferably below 1.0% EA, whatever source, will minimise the threat. Examination of old farm records, particularly from the AAPS Industrial Cropping on Set-aside era may identify previously unsuspected threats on farms, particularly where new land has been acquired, or fields merged. While not identified as contributory to elevated erucic acid within the set of project samples, brassica weeds remain a threat to crop quality if not controlled. Development of a soil/weed test to assess the risk from volunteers in fields further down the rotation may provide a useful predictive test on heavily infested land. Growing Clearfield® varieties and managing volunteers and brassica weeds with the associated products provides a useful further strategy when used as part of an integrated approach to managing weeds and volunteers across the crop rotation.

In addition to cultural practices, it is vital to maintain very good record keeping of grain movements on and off farms, to avoid and mixing of HEAR and LEAR seed. Reference samples from individual loads should be kept as a matter of routine, to be used in the event of any disputed test results.

Given the apparently widespread incidence of volunteers with elevated levels of erucic acid, it is recommended that consideration is given to introducing routine EA tests, as an addition to the current seed certification scheme for oilseed rape varieties, using the samples already collected for seed purity analysis.

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8. Appendices

8.1 Comparison of erucic acid testing methodology by Perkin Elmer Clarus 600 gas chromatography, FOSS DA 1650 NIRS and Infratech 1241 NIRS

8.2 Weed seed contamination of 25 samples of oilseed rape from Harvest 2017

8.3 50 x single seed erucic acid analyses

8.4 Erucic acid content of 915 commercial samples of oilseed rape after harvest 2018

8.1 Comparison of erucic acid testing methodology by Perkin Elmer Clarus 600 gas chromatography, FOSS DA 1650 NIRS and Infratech 1241 NIRS

Sample	Perkin Elmer 600 GC			FOSS DA 1650 NIRS			Infratech 1241 NIRS			
number	Rep 1	Rep 2	**Mean**	Rep 1	Rep 2	**Mean**	Rep 1	Rep 2	Rep 3	**Mean**
1	7.49	8.23	**7.86**	5.62	6.29	**5.96**	29.24	25.07	27.48	**27.26**
2	5.10	3.32	**4.21**	3.51	3.20	**3.36**	18.85	16.71	12.26	**15.94**
3	1.67	1.64	**1.66**	0.59	1.83	**1.21**	9.61	8.48	17.53	**11.87**
4	2.09	2.55	**2.32**	2.52	0.83	**1.68**	8.63	11.88	10.43	**10.31**
5	0.07	0.07	**0.07**	0.14	0.61	**0.38**	17.33	12.89	12.18	**14.13**
6	4.90	5.66	**5.28**	3.56	3.46	**3.51**	13.10	12.18	7.85	**11.04**
7	0.12	0.16	**0.14**	0.00	0.00	**0.00**	5.09	8.87	10.15	**8.04**
8	1.28	1.12	**1.20**	0.91	2.37	**1.64**	3.52	10.13	13.14	**8.93**
9	3.68	4.81	**4.25**	3.89	3.46	**3.68**	17.91	15.30	9.92	**14.38**
10	0.89	1.32	**1.11**	2.94	2.55	**2.75**	9.29	6.21	8.88	**8.13**
11	1.40	1.77	**1.59**	1.07	1.48	**1.28**	9.12	14.46	13.02	**12.20**
12	1.07	1.23	**1.15**	2.30	1.37	**1.84**	9.04	7.53	6.80	**7.79**
13	1.61	1.20	**1.41**	2.37	1.96	**2.17**	10.73	8.08	11.75	**10.19**
14	15.70	15.91	**15.81**	11.63	12.57	**12.10**	23.16	27.90	22.25	**24.44**
15	3.50	4.14	**3.82**	4.50	3.70	**4.10**	11.74	9.43	15.23	**12.13**
16	1.24	3.95	**2.60**	2.91	2.39	**2.65**	9.57	14.72	25.28	**16.52**
17	4.14	3.90	**4.02**	5.40	4.00	**4.70**	10.39	13.58	11.37	**11.78**
18	0.28	0.40	**0.34**	0.95	0.00	**0.48**	7.38	9.76	10.13	**9.09**
19	1.24	1.61	**1.43**	2.48	2.22	**2.35**	9.10	11.99	9.15	**10.08**
20	2.27	2.42	**2.35**	5.13	3.92	**4.53**	11.80	16.16	12.69	**13.55**
21	1.97	2.04	**2.01**	2.67	1.53	**2.10**	10.44	10.76	15.83	**12.34**
22	0.17	0.05	**0.11**	0.97	0.91	**0.94**	6.97	9.15	13.72	**9.95**
23	4.27	5.00	**4.64**	8.04	6.13	**7.09**	7.80	5.26	7.23	**6.76**
24	7.32	7.47	**7.40**	8.80	11.63	**10.22**	13.62	5.96	8.24	**9.27**
25	2.02	1.97	**2.00**	3.70	3.95	**3.83**	-2.97	9.29	5.52	**3.95**
26	3.41	3.67	**3.54**	3.47	3.53	**3.50**	7.00	3.56	4.68	**5.08**
27	9.07	9.09	**9.08**	6.17	7.38	**6.78**	19.68	19.20	21.58	**20.15**
28	20.07	19.69	**19.88**	23.48	21.28	**22.38**	16.69	17.52	16.44	**16.88**
29	0.34	0.17	**0.26**	0.03	0.00	**0.02**	5.44	11.69	12.05	**9.73**
30	0.06	0.06	**0.06**	0.00	0.23	**0.12**	9.34	7.11	13.47	**9.97**
31	3.41	2.86	**3.14**	3.21	3.42	**3.32**	14.41	13.20	13.77	**13.79**
32	0.09	0.14	**0.12**	0.00	0.70	**0.35**	-0.61	12.78	1.93	**4.70**
33	0.16	0.10	**0.13**	0.00	0.00	**0.00**	7.12	4.04	13.21	**8.12**
34	2.18	3.30	**2.74**	4.12	4.69	**4.41**	11.10	10.45	8.45	**10.00**
35	1.60	1.90	**1.75**	0.47	0.35	**0.41**	8.44	5.41	10.83	**8.23**
36	5.59	8.32	**6.96**	8.19	7.69	**7.94**	6.64	6.61	7.41	**6.89**
37	3.19	3.29	**3.24**	3.59	3.69	**3.64**	7.11	10.47	1.97	**6.52**
38	0.23	0.22	**0.23**	0.15	0.00	**0.08**	10.90	7.68	11.69	**10.09**
39	0.16	0.07	**0.12**	0.00	0.00	**0.00**	19.28	23.14	21.83	**21.42**
40	0.12	0.16	**0.14**	0.00	0.00	**0.00**	26.95	24.29	25.17	**25.47**
41	0.18	0.38	**0.28**	0.30	0.91	**0.61**	5.71	6.19	8.08	**6.66**
42	5.11	5.29	**5.20**	3.80	2.71	**3.26**	11.48	20.49	23.44	**18.47**
43	35.53	36.06	**35.80**	31.55	32.27	**31.91**	30.78	34.48	34.89	**33.38**
44	1.90	1.57	**1.74**	0.00	0.00	**0.00**	6.28	8.44	8.77	**7.83**
45	0.05	0.02	**0.04**	0.00	0.00	**0.00**	2.54	2.62	3.78	**2.98**
46	2.25	3.01	**2.63**	4.18	4.50	**4.34**	16.06	18.33	12.57	**15.65**
47	0.10	0.11	**0.11**	0.00	0.00	**0.00**	-4.95	-1.62	-1.53	**-2.70**
48	2.16	1.81	**1.99**	2.04	1.89	**1.97**	10.25	5.03	11.13	**8.80**
49	0.92	1.20	**1.06**	1.09	0.55	**0.82**	6.55	2.49	3.57	**4.20**
50	0.03	0.06	**0.05**	0.00	0.00	**0.00**	-6.99	-1.00	-2.63	**-3.54**
Mean	3.47	3.69	3.58	3.65	3.56	3.61	10.55	11.49	12.09	11.38
LSD			0.97			1.25				5.12

8.2. Weed seed analysis by species and percent weight of original sample (100g)

Species	Sample number																								
	1	2	4	6	8	14	23	24	27	28	30	31	32	33	36	37	39	41	42	43	44	45	46	48	50
Aethusa cynapium		0.01	0.01			0.01		0.01														0.01			
Alliaria petiolata		0.01																							
Alopecurus myosuroides	0.39	0.17	0.01									0.10						0.01	0.01		0.01		0.01		
Anchusa arvensis																						0.01			
Anthemis cotula										0.01															
Anthriscus sp.								0.01																	
Anthriscus sylvestris	0.05	0.30											0.01			0.01									
Avena fatua																					0.01				
Barbarea vulgaris	0.06	0.01								0.09			0.01												
Brassica juncea/Brassica rapa																								0.09	
Brassica nigra																		0.32							
Bromus hordeaceus													0.01												
Bromus sterilis								0.01				0.10	0.01												
Capsella bursa-pastoris	0.01	0.01			0.01	0.01	0.01				0.01	0.01	0.13	0.01					0.01						
Chenopodium album		0.01						0.01		0.01			0.01												
Cirsium vulgare																0.01									
Conium maculatum																							0.01		
Dactylis glomerata													0.01												
Elytrigia repens													0.01												
Erodium cicutarium																						0.01			
Festuca rubra/ovina													0.01			0.01									
Galium aparine	0.38	0.58	0.05		0.07		0.01	0.05		0.01		0.72	0.01				0.01	0.06	0.01	0.01	0.01	0.10	0.81	0.01	0.01
Geranium dissectum	0.06	0.01	0.01			0.01		0.01		0.08		0.05	0.01	0.01		0.31		0.12	0.06	0.01	0.01	0.01	0.09	0.01	
Geranium molle																							0.01		
Geranium pusillum		0.01											0.01				0.01					0.01	0.01		
Hordeum vulgare		0.05					0.01	0.10														0.21			
Hypericum hirsutum																0.01									
Lactuca sativa												0.04													
Lapsana communis					0.01			0.01					0.01						0.01				0.01		
Linum usitatissimum				0.01																					
Lolium sp.											0.01														
Matricaria chamomilla											0.01														

8.2 Weed seed analysis (continued)

Species	Sample number																							
	1	2	4	6	8	14	23	24	27	28	30	31	32	33	36	37	39	41	42	43	44	45	46	48	50
Matricaria discoidea													0.01												
Myosotis arvensis													0.01												
Papaver sp.						0.01	0.01		0.01			0.01	0.01												
Petroselinum crispum								0.01																	
Phleum sp.													0.01												
Poa annua											0.01		0.01												
Poa trivialis													0.01												
Polygonum aviculare													0.01												
Raphanus raphanistrum								0.01																	
Rumex acetosella											0.01														
Rumex crispus			0.01																				0.01		
Rumex obtusifolius		0.01											0.01						0.01						
Seed of Asteraceae										0.01															
Senecio vulgaris								0.01					0.01						0.01						
Silene latifolia																			0.01			0.01			
Silene noctiflora																					0.01				
Silene sp.											0.01														
Sinapis arvensis		0.01			0.06	0.01						0.04						0.01							
Sisymbrium officinale		0.01				0.01	0.01	0.01		0.01		0.02	1.12	0.01				0.01	0.01	0.01			0.01		
Sonchus asper						0.01				0.01									0.01			0.01			
Sonchus oleraceus		0.01																							
Stellaria media					0.01						0.01	0.03	0.01									0.01			
Thlaspi arvense	0.01	0.01																							
Torilis nodosa						0.01		0.01	0.01									0.01							
Tripleurospermum inodorum						0.01							0.01												
Triticum aestivum																0.01							0.01		
Veronica persica													0.01										0.01		
Veronica sp.								0.01																	
Viola sp.							0.01						0.01												
Vulpia sp.								0.01																	
All weed seed % weight	1.95	3.16	4.07	6.01	8.15	14.05	23.03	24.22	27.01	28.21	30.04	32.12	33.37	33.02	36.00	37.34	39.01	41.52	42.10	43.02	44.03	45.35	46.94	48.10	50.01

8.3 50 x single seed erucic acid tests - in ascending order - high values highlighted

	Sample number (selected from original 1-50 set)											
Seed no.	**1**	**6**	**14**	**23**	**24**	**27**	**28**	**36**	**37**	**42**	**43**	**48**
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00
5	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00
6	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00
7	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00
8	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00
9	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00
10	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.95	0.00
11	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.00	8.29	0.00
12	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.00	11.42	0.00
13	0.00	0.00	0.02	0.00	0.00	0.00	0.03	0.00	0.00	0.00	12.86	0.00
14	0.02	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.00	19.35	0.00
15	0.02	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.00	19.37	0.00
16	0.02	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.00	22.45	0.00
17	0.02	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.00	24.46	0.00
18	0.02	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.00	25.04	0.00
19	0.02	0.00	0.04	0.00	0.00	0.00	0.04	0.00	0.00	0.00	26.00	0.00
20	0.02	0.00	0.04	0.00	0.00	0.00	0.04	0.01	0.00	0.00	26.15	0.00
21	0.02	0.00	0.04	0.00	0.00	0.00	0.04	0.02	0.00	0.00	26.70	0.00
22	0.02	0.00	0.04	0.00	0.00	0.00	0.04	0.02	0.00	0.00	26.83	0.00
23	0.02	0.00	0.05	0.00	0.00	0.00	0.04	0.02	0.00	0.00	33.84	0.00
24	0.02	0.02	0.06	0.00	0.00	0.00	0.05	0.02	0.00	0.00	35.28	0.00
25	0.02	0.02	0.06	0.00	0.00	0.00	0.05	0.02	0.00	0.00	37.18	0.00
26	0.03	0.04	0.07	0.00	0.00	0.00	0.06	0.03	0.00	0.00	39.04	0.00
27	0.03	0.04	0.07	0.00	0.01	0.02	0.08	0.04	0.00	0.00	39.16	0.00
28	0.03	0.04	0.09	0.00	0.01	0.03	0.08	0.04	0.00	0.00	39.26	0.00
29	0.05	0.04	0.09	0.00	0.01	0.03	0.10	0.04	0.00	0.00	39.65	0.00
30	0.05	0.05	0.11	0.00	0.02	0.03	10.29	0.04	0.00	0.00	40.50	0.00
31	0.06	0.05	0.11	0.00	0.02	0.04	12.27	0.04	0.00	0.00	40.84	0.00
32	0.07	0.05	0.12	0.00	0.02	0.04	28.93	0.05	0.00	0.00	40.96	0.00
33	0.08	0.06	16.33	0.00	0.03	0.05	32.40	0.05	0.00	0.00	42.34	0.00
34	0.10	0.08	18.87	0.00	0.03	0.06	40.48	0.07	0.00	0.01	42.44	0.00
35	0.10	0.09	23.67	0.01	0.03	0.06	40.75	0.07	0.00	0.02	42.68	0.00
36	0.13	0.10	29.99	0.01	0.03	0.08	42.29	0.15	0.00	0.04	44.01	0.00
37	0.13	0.11	31.48	0.02	0.04	25.91	42.95	0.20	0.00	0.05	44.48	0.00
38	0.13	0.12	33.44	0.02	0.04	26.97	44.64	10.51	0.00	0.06	44.48	0.00
39	15.74	13.05	34.03	0.02	0.04	27.16	45.81	26.47	0.00	0.16	44.91	0.00
40	29.52	17.46	37.14	0.02	0.05	29.67	48.33	32.19	0.00	13.64	45.11	0.00
41	33.56	31.99	38.95	0.02	0.05	31.28	48.46	39.33	0.00	27.89	45.49	0.00
42	40.15	32.32	39.51	0.03	27.88	37.28	50.17	44.52	0.00	30.30	45.49	0.00
43	43.53	32.59	40.91	0.03	34.72	40.25	50.37	44.85	0.01	32.85	45.76	0.00
44	43.72	33.70	42.25	0.03	41.86	42.30	50.80	45.57	0.02	41.69	45.99	0.00
45	45.80	42.41	42.97	0.04	42.26	43.11	51.55	46.73	0.03	44.91	46.45	0.00
46	47.62	42.91	43.68	0.05	42.48	44.40	51.68	47.14	0.05	45.08	47.36	0.00
47	48.24	47.17	43.85	16.82	43.36	45.30	52.27	48.34	26.87	49.71	47.71	0.00
48	48.67	47.19	44.32	28.12	45.29	45.87	52.68	48.62	39.24	51.52	47.97	0.00
49	51.40	47.33	44.62	31.88	45.70	46.25	54.03	49.24	41.83	53.18	49.62	0.00
50	52.60	49.97	44.63	41.71	50.16	49.97	54.77	49.69	43.69	53.45	52.36	50.08
Mean EA%	10.04	8.78	13.04	2.38	7.48	10.72	18.14	10.68	3.03	8.89	29.22	1.00
Bulk EA%	7.86	5.28	15.81	4.64	7.40	9.08	19.88	6.96	3.24	5.20	35.80	1.99

Appendix 8.4

Figure 10. Erucic acid content (%) in 915 commercial samples of oilseed rape after harvest 2018, presented in erucic acid categories and as a percent of the total