Investigation into the emerging problem of elevated erucic acid content in double-low oilseed rape crops in the UK

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Simon Kightley, Helen Appleyard, Pravina Solanki,
David Evershed, Linda Maile, Tom Wood,
Rosa Caiazzo, Will Smith

The problem

- Worrying proportion of rapeseed loads reported to be above the current 5% erucic acid threshold
- Moving to a new 2% threshold in the oil will exacerbate this
- Crushers identifying elevated erucic acid in the oil
- Farmers penalized
- Until recently, no reliable rapid test for EA.

Abbreviations

- EA – erucic acid
- HEAR – high erucic acid rape
- LEAR – low erucic acid rape
- GC – gas chromatography
- NIRS – near infra red spectroscopy
- OSR oilseed rape

Possible causes

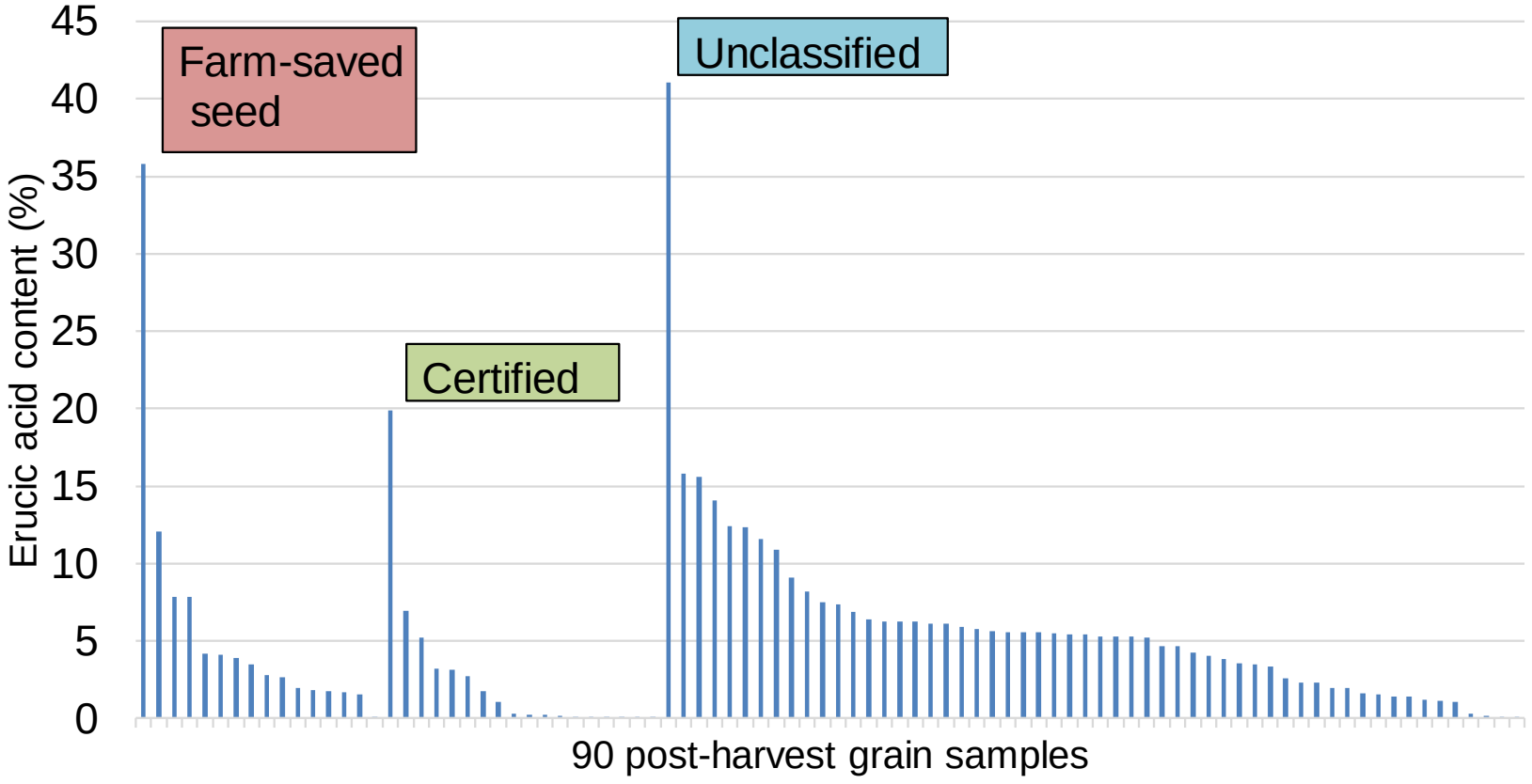
- In field contamination
  - Pollen drift from nearby HEAR crops (~20,000ha)
  - Volunteers – HEAR or LEAR/HEAR crosses – recent or historic
  - Reducing OSR seed rates
  - Oil-bearing weed seeds (e.g.: charlock)
  - Inaccurate testing at intake
  - Contaminated sown seed – farm-saved or certified
- Occasional human error during :
  - Seeding
  - Harvest
  - Storage
  - Transport

The approach - using a set of 50 grain samples from Harvest 2017:

- Examine GC:NIRS calibration for EA content
- Clean 25 samples to remove and classify all weed seeds to examine this cause of contamination
- Forensic examination of 12 ‘cleaned’ samples by testing EA content of 50 single seeds to determine whether elevated EA levels are ‘variety drift’ or contamination

Initial screening of 90 harvest samples

Erucic acid content of 90 crop samples received arranged by sown seed type

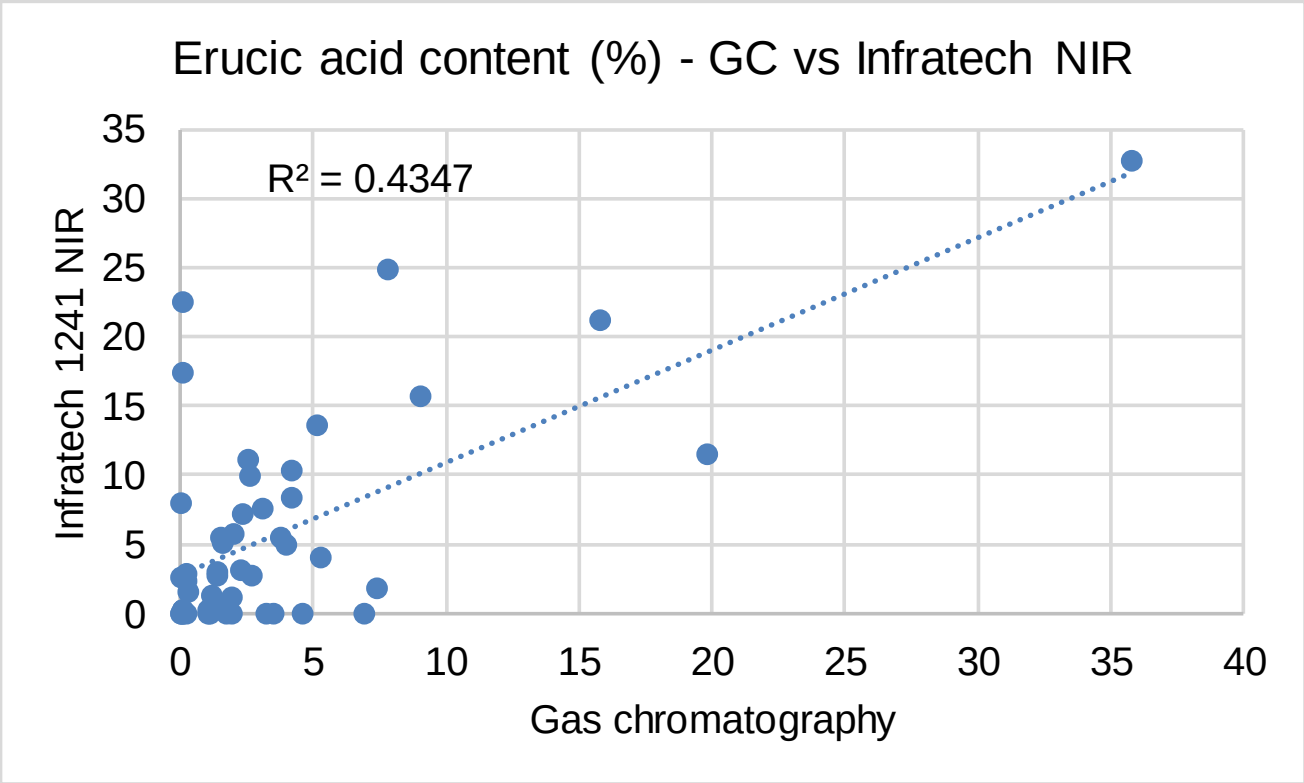


Provisional conclusions from EA analysis of 90 samples

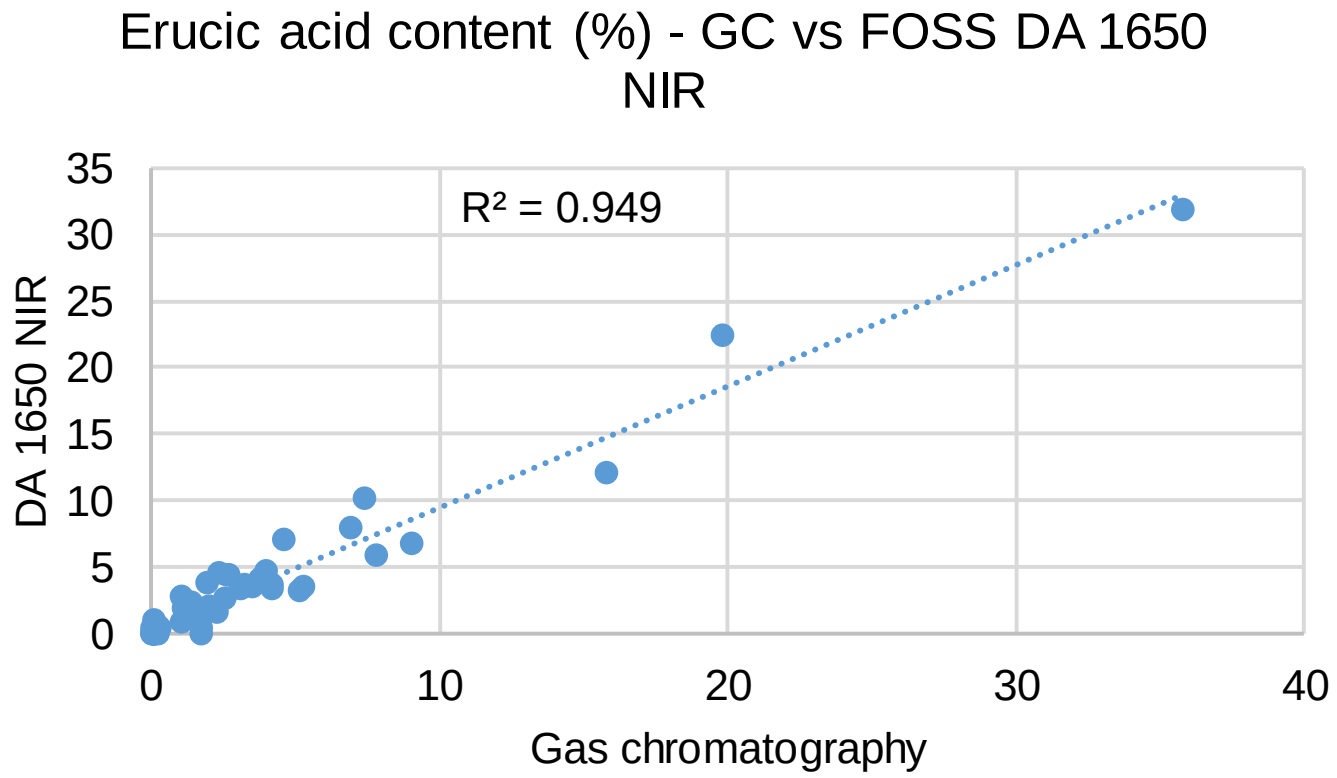
- Farm saved seed carries more risk than certified seed
- Conventional, line varieties carry more risk than hybrids

Sample testing

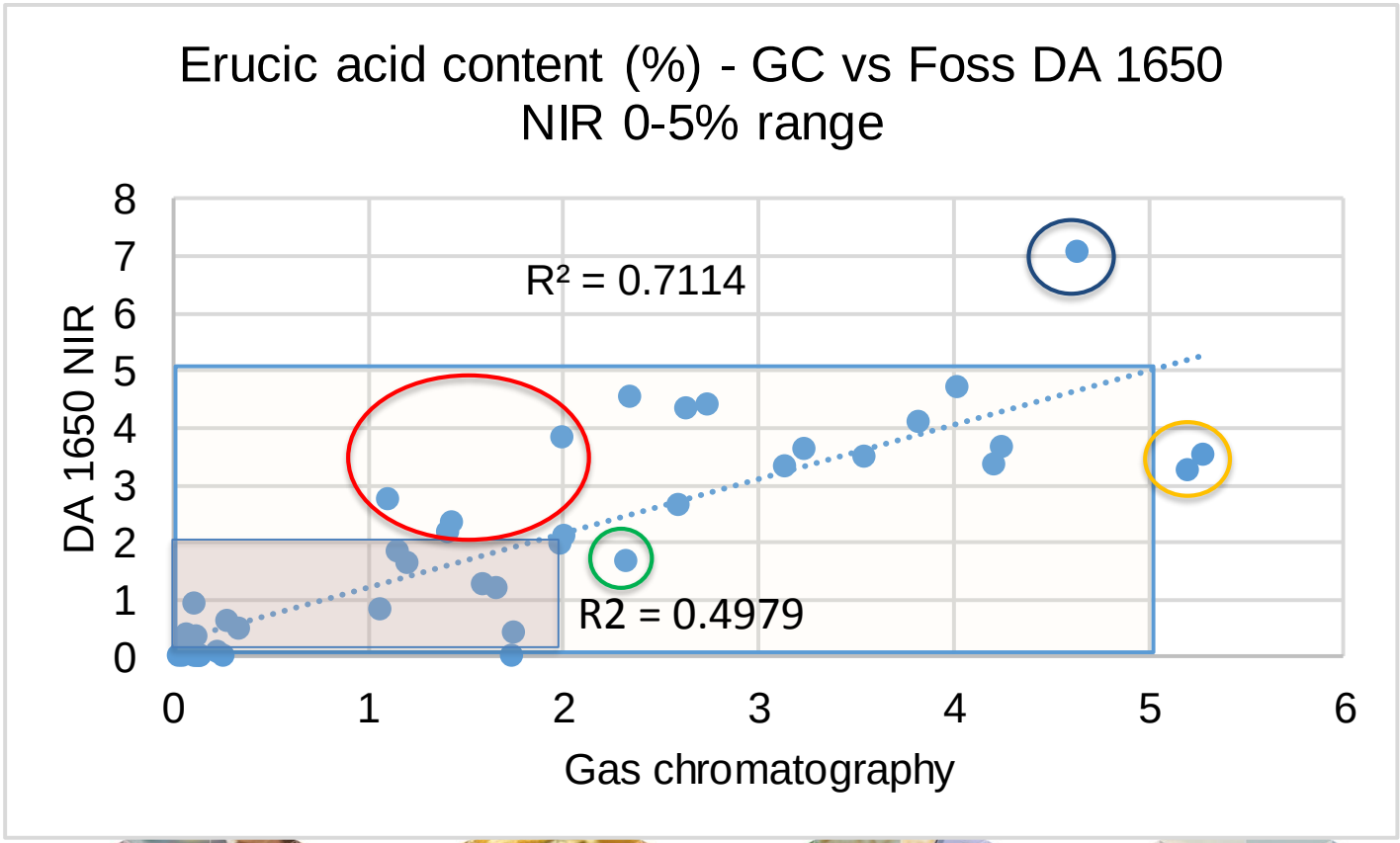
Old technology: GC vs Infratech 1241 NIRS



New technology: Gas chromatography vs Foss DA 1650 NIRS



Less precision at lower thresholds

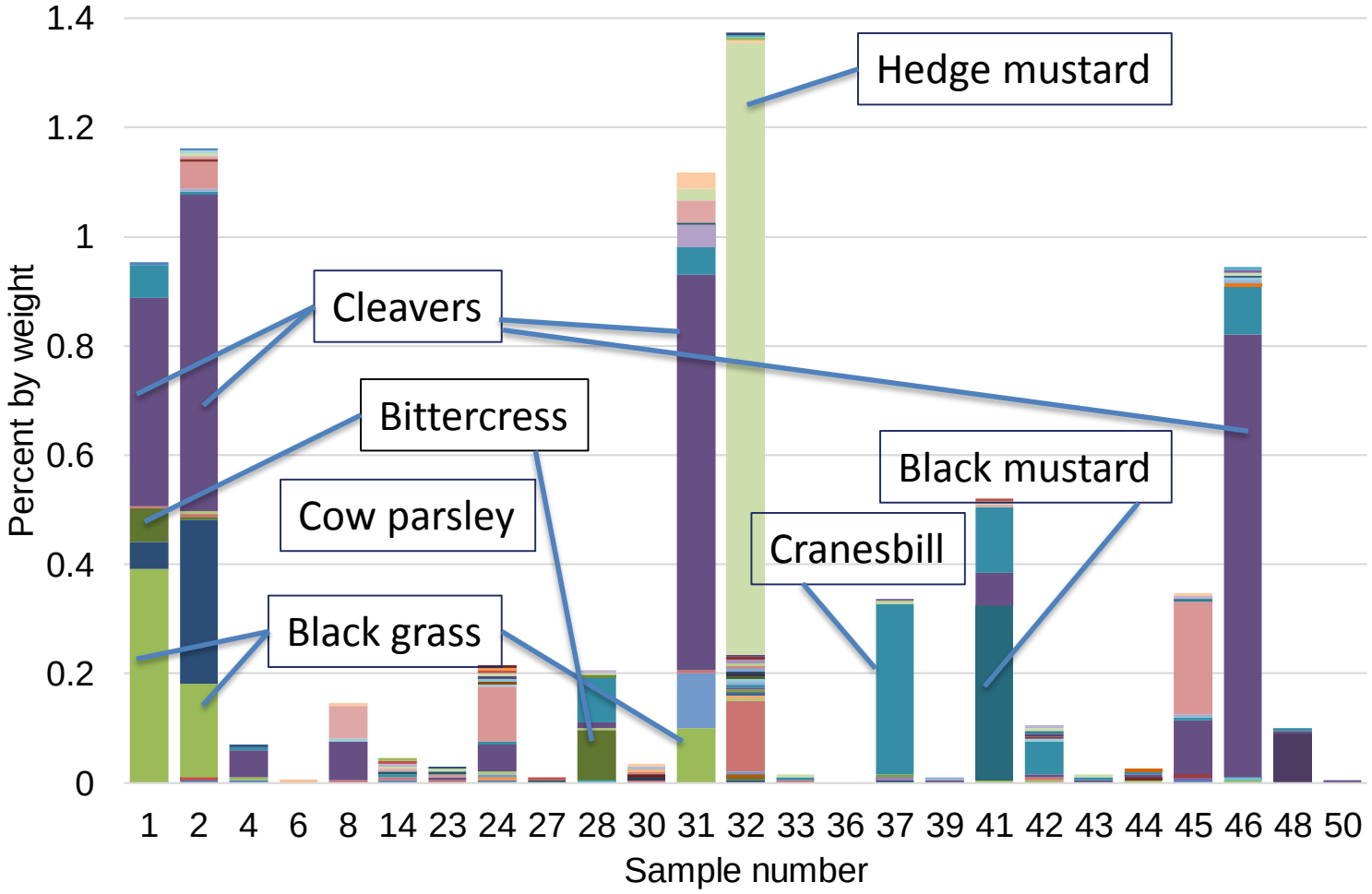


Conclusions on seed testing

- NIRS can provide a reasonably accurate rapid test for erucic acid in rape.
- At the 2% EA threshold, our evidence suggests that it will over-estimate the EA in a proportion of tests.

The influence of weed seeds

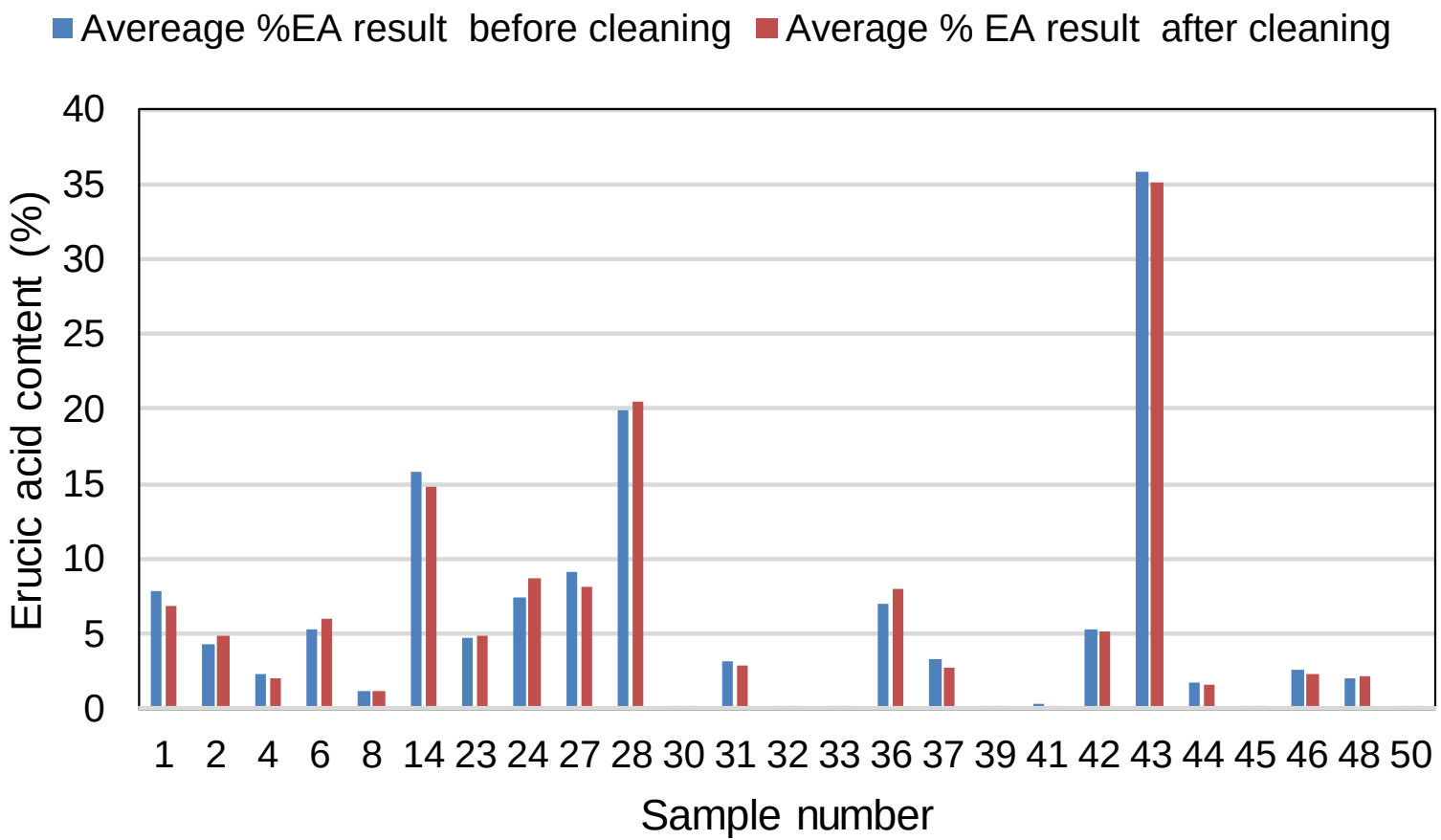
Sample purity examinations (25 samples)



Erucic acid content in weed seed oil profiles (%)

Barbarea vulgaris Sample 1	Bittercress	46.02
Barbarea vulgaris Sample 28	Bittercress	24.52
Sinapsis arvensis Sample 8	Charlock	42.53
Brassica rapa Sample 48	Turnip rape	41.09
Brassica nigra Sample 41	Black mustard	36.99
Sisymbrium officinale 32	Hedge mustard	23.89
Capsella bursa pastons Sample 32	Shepherds purse	0.36
Galium aparine 1, 2, 31	Cleavers	0.18
Alopecurus myosuriodes 1 and 2	Black grass	0.00

Erucic acid content before and after cleaning



Conclusions from sample purity examinations

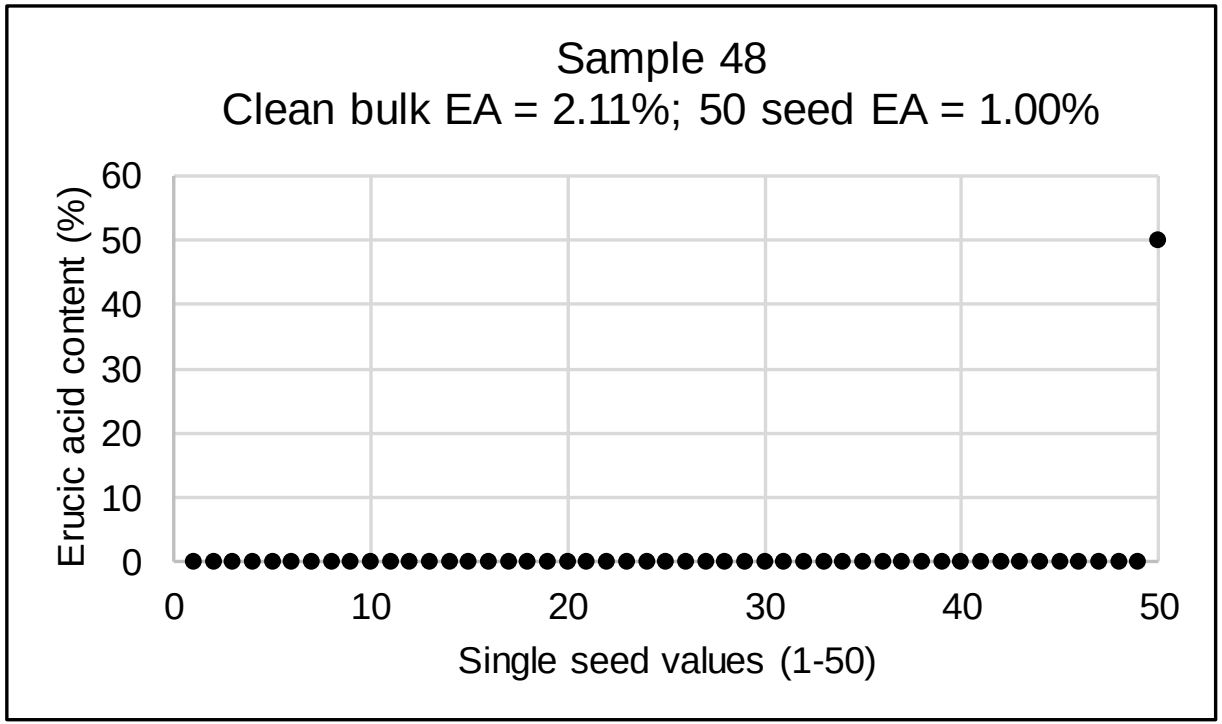
- The erucic acid levels in NONE of these samples were significantly influenced by the presence of weed seed.

- This does not rule out contamination by uncontrolled Brassica weed infestations in other situations.

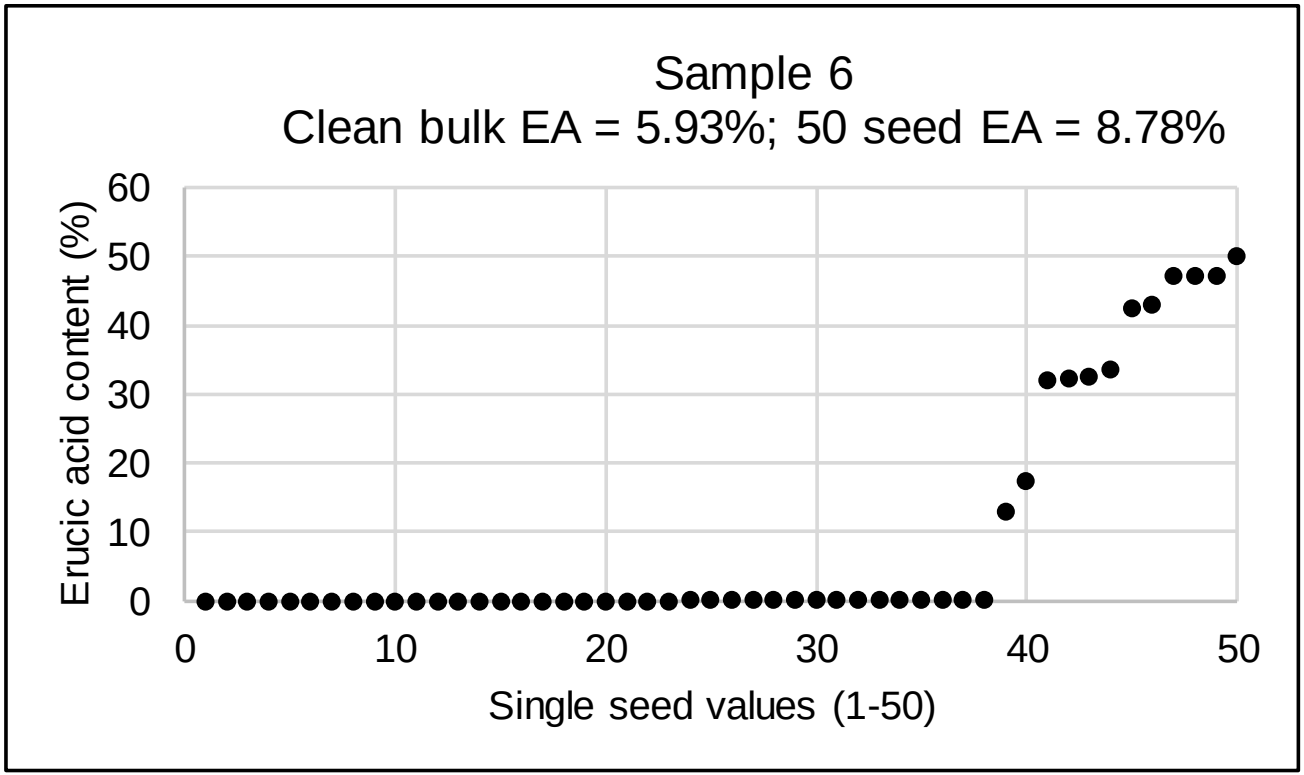
Single seed tests

- Perkin Elmer Clarus 600 chromatograph
- 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
- For these tests 0.2ml heptane/seed was used for extraction and the assay was adapted to split-less injection into the GC column.

The simplest possible example



A more complex sample



Our assumptions

Oilseed rape has two genomes (*B. rapa and B. oleracea.*) i.e.: 2 sets of genes. For EA control:

Dominant and recessive *Bn-FAE1.1* and *BnFAE1.2* gene loci, represented by ‘A’ and ‘a’ and ‘B’ and ‘b’

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

Cross pollination over years and multiple rotations

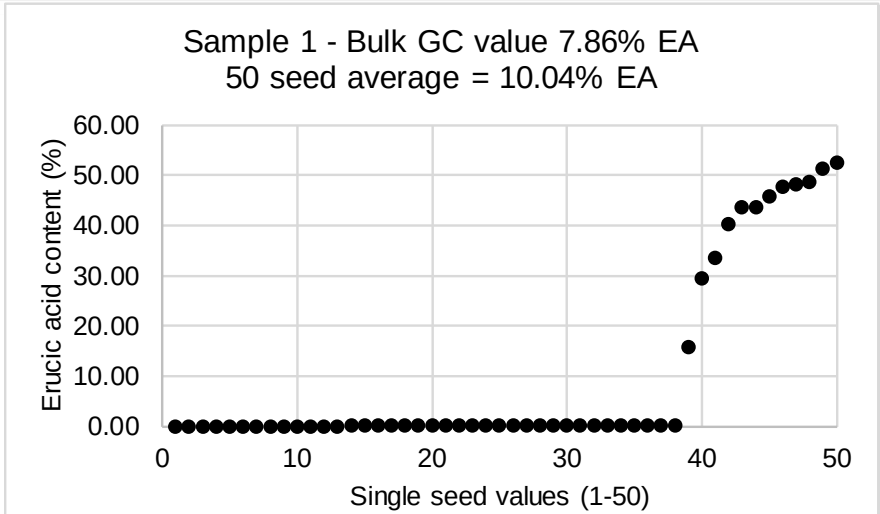
In the second generation of crossing, when fields come back into oilseed rape, we will have five crossing outcomes:

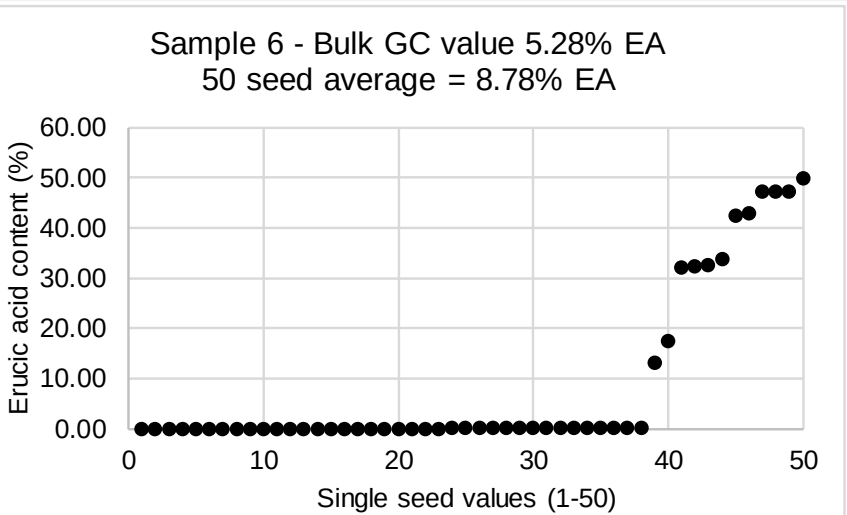
AA BB ~ 45-50% EA
AA Bb ~ 35-40% EA
Aa Bb ~ 22-27% EA
aa bB ~10-15% EA
aa bb <0.1% EA

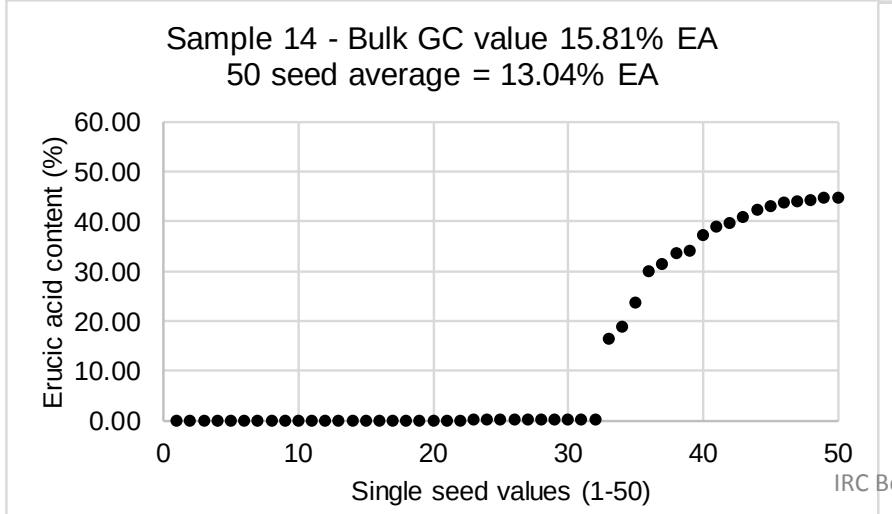
The proportions of all five will be determined by whatever emerges that season, drilled crop plants and volunteers.

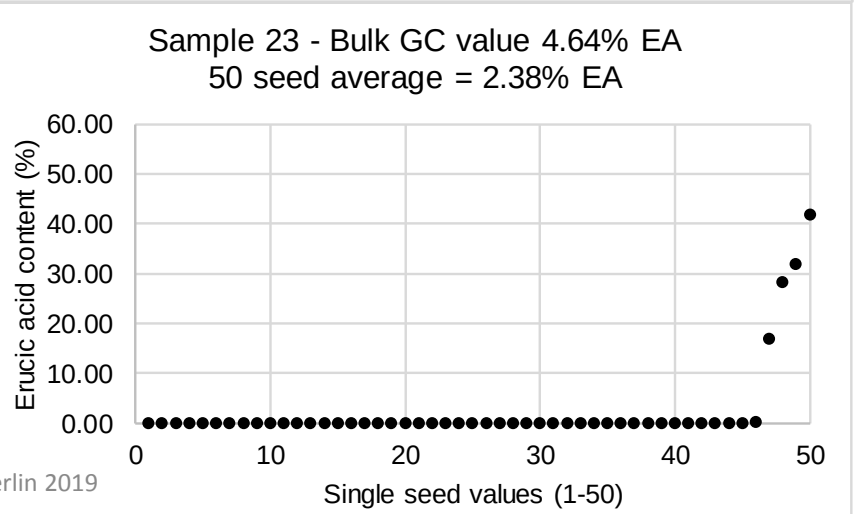
16 theoretical outcomes for erucic acid determination

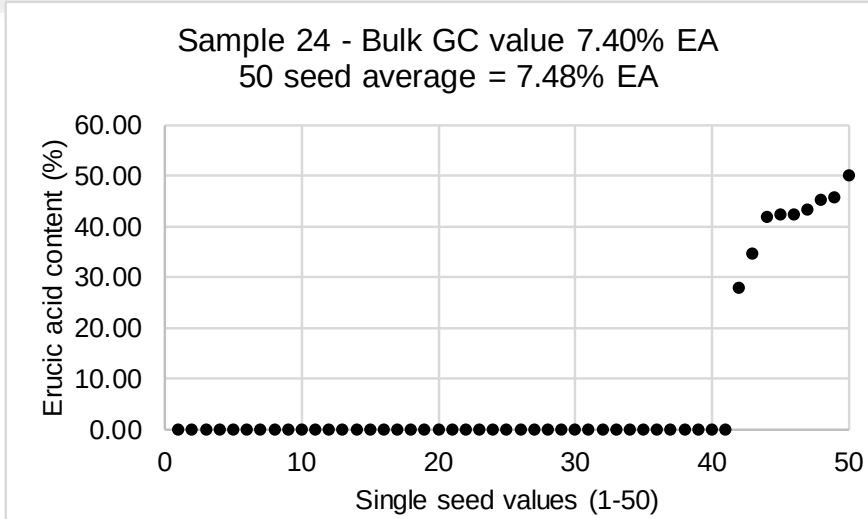
		Pollen (Haploid)			
		AB	Ab	aB	ab
Recipient plant ovule (haploid)	AB	AABB High (~50%)	AABb Intermediate-high (37.5%)	AaBB Intermediate-high (37.5%)	AaBb Intermediate (~25%)
	Ab	AA Bb Intermediate-high (~37%)	AAbb Intermediate (~25%)	AaBb Intermediate (~25%)	Aabb Intermediate-low (~12.5%)
	aB	AaBB Intermediate-high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

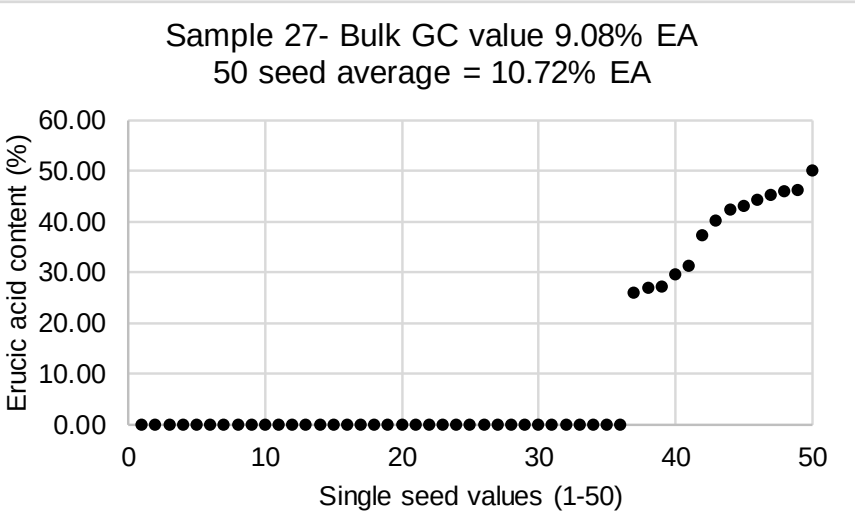

Sample 1 - Bulk GC value 7.86% EA
50 seed average = 10.04% EA
Erucic acid content (%)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
10
20
30
40
50
Single seed values (1-50)

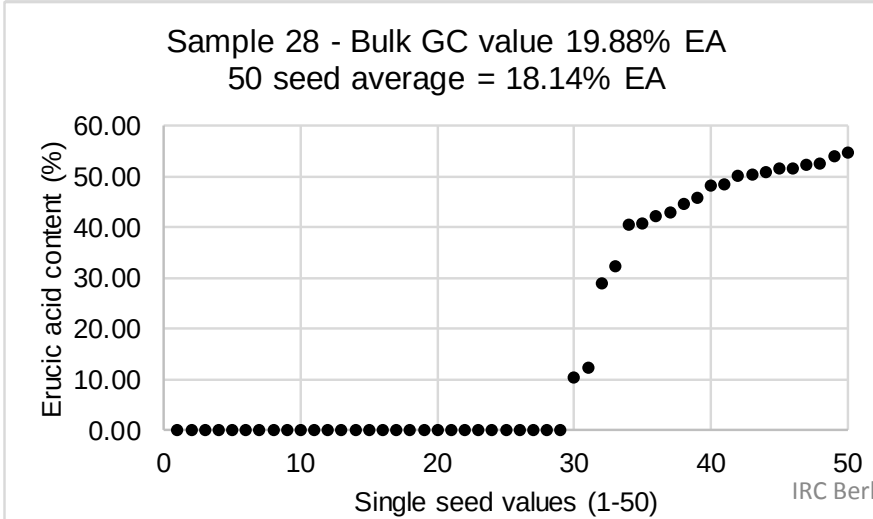

Sample 6 - Bulk GC value 5.28% EA
50 seed average = 8.78% EA
Erucic acid content (%)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
10
20
30
40
50
Single seed values (1-50)

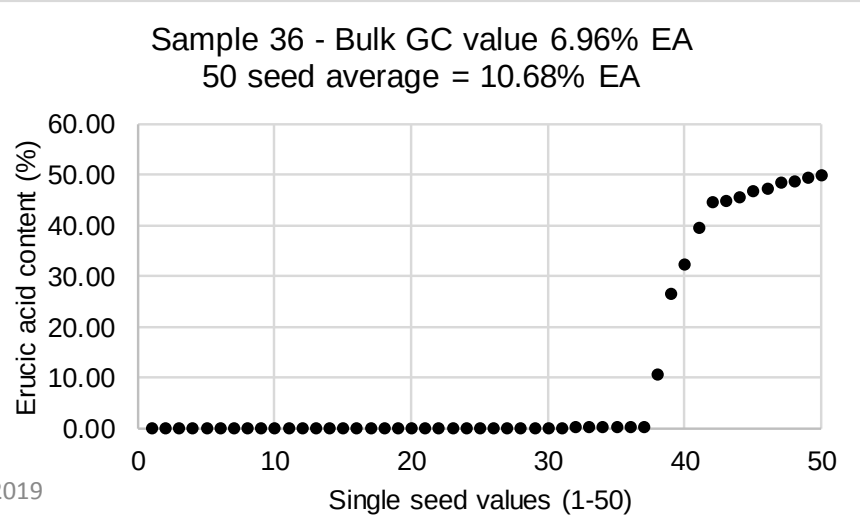

Sample 14 - Bulk GC value 15.81% EA
50 seed average = 13.04% EA
Erucic acid content (%)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
10
20
30
40
50
Single seed values (1-50)

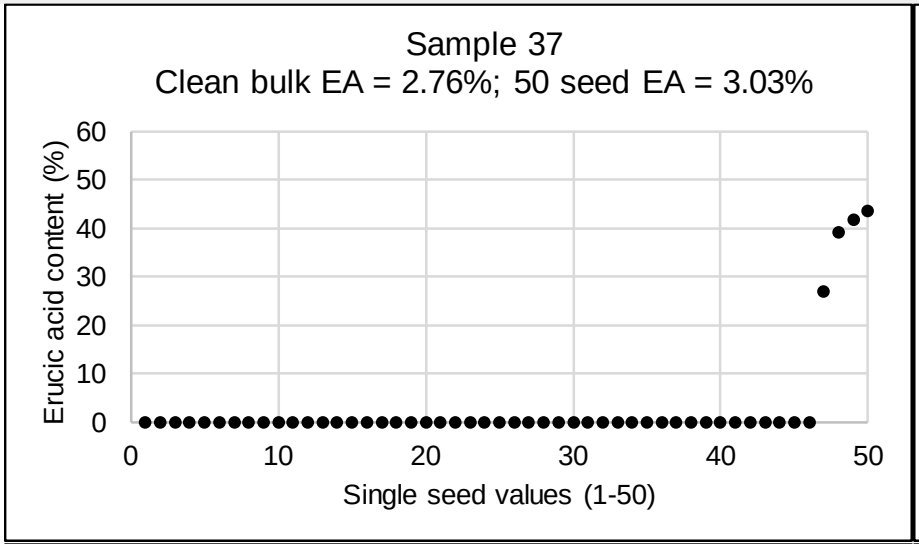

Sample 23 - Bulk GC value 4.64% EA
50 seed average = 2.38% EA
Erucic acid content (%)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
10
20
30
40
50
Single seed values (1-50)

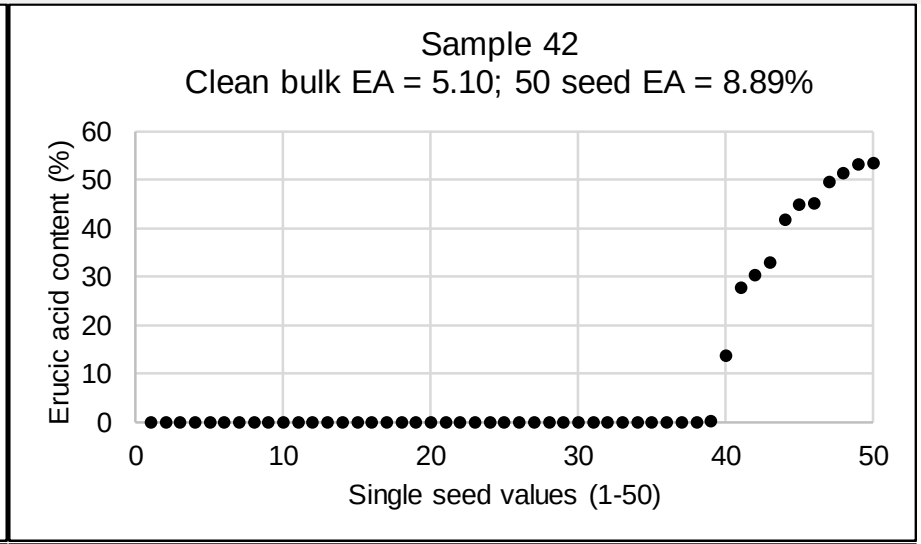

Sample 24 - Bulk GC value 7.40% EA
50 seed average = 7.48% EA
Erucic acid content (%)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
10
20
30
40
50
Single seed values (1-50)

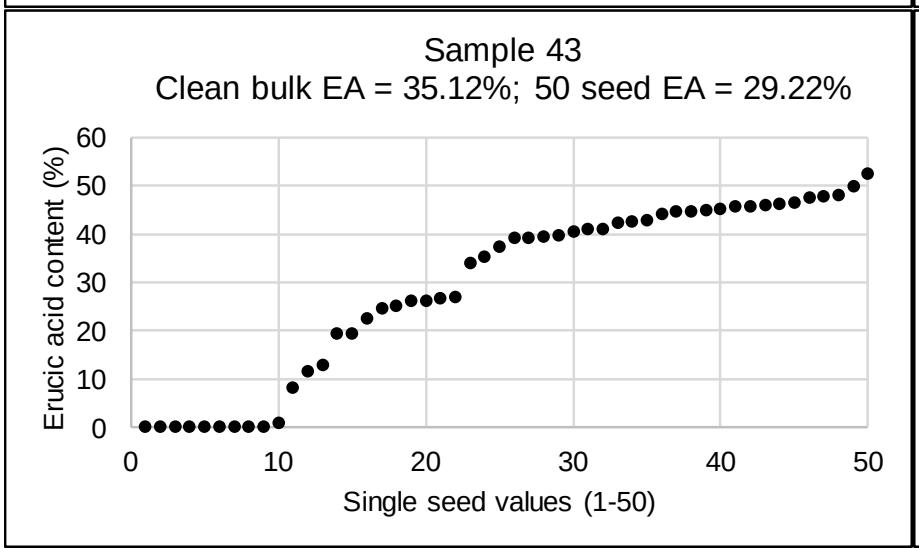

Sample 27- Bulk GC value 9.08% EA
50 seed average = 10.72% EA
Erucic acid content (%)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
10
20
30
40
50
Single seed values (1-50)

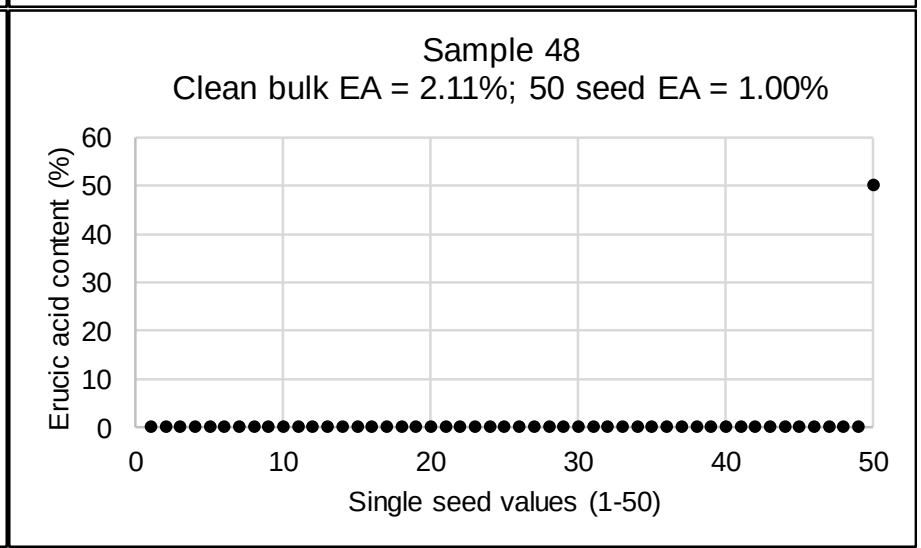

Sample 28 - Bulk GC value 19.88% EA
50 seed average = 18.14% EA
Erucic acid content (%)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
10
20
30
40
50
Single seed values (1-50)


Sample 36 - Bulk GC value 6.96% EA
50 seed average = 10.68% EA
Erucic acid content (%)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
10
20
30
40
50
Single seed values (1-50)

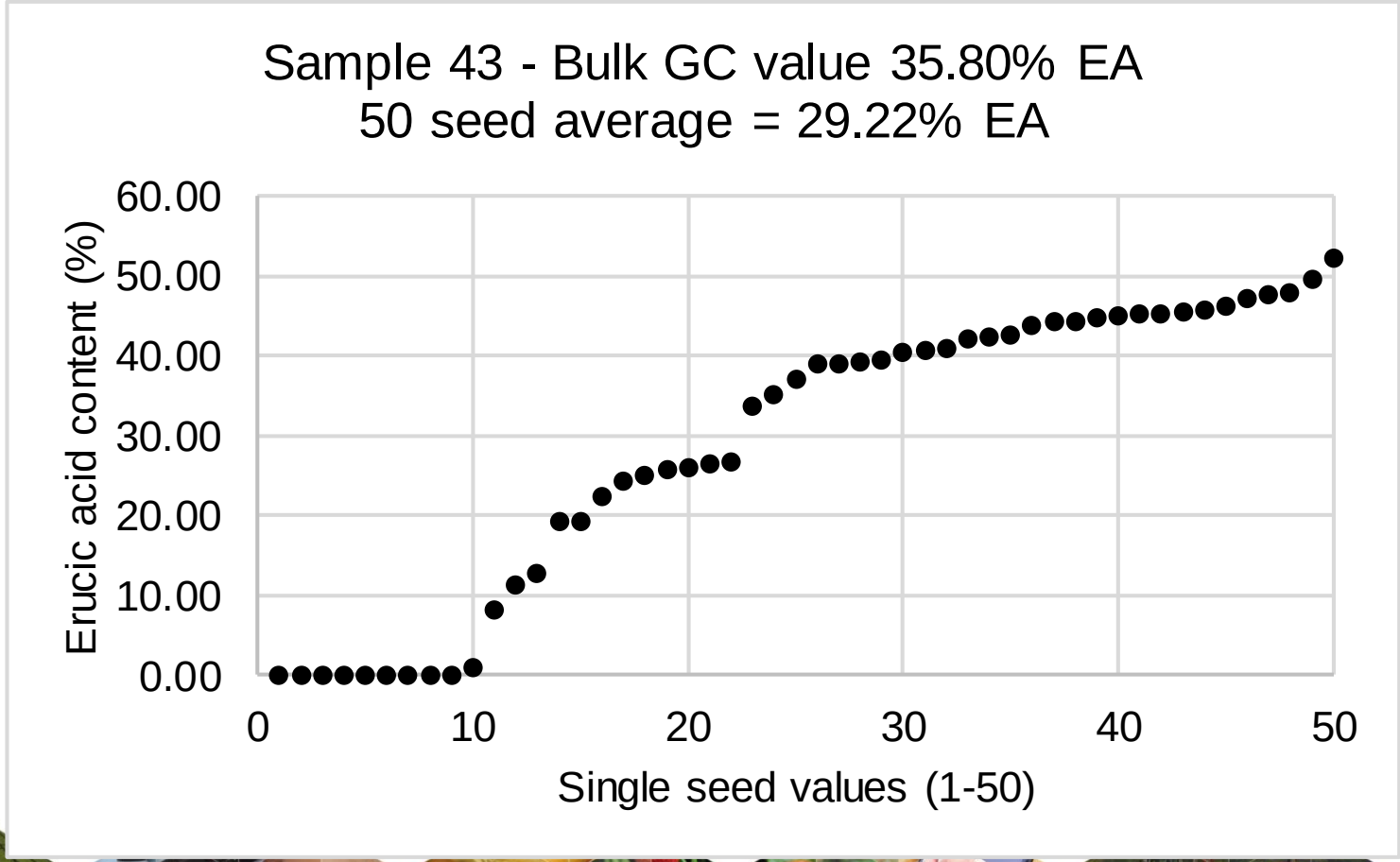

Sample 37
Clean bulk EA = 2.76%; 50 seed EA = 3.03%
Erucic acid content (%)
0
10
20
30
40
50
60
0
10
20
30
40
50
Single seed values (1-50)


Sample 42
Clean bulk EA = 5.10; 50 seed EA = 8.89%
Erucic acid content (%)
0
10
20
30
40
50
60
0
10
20
30
40
50
Single seed values (1-50)

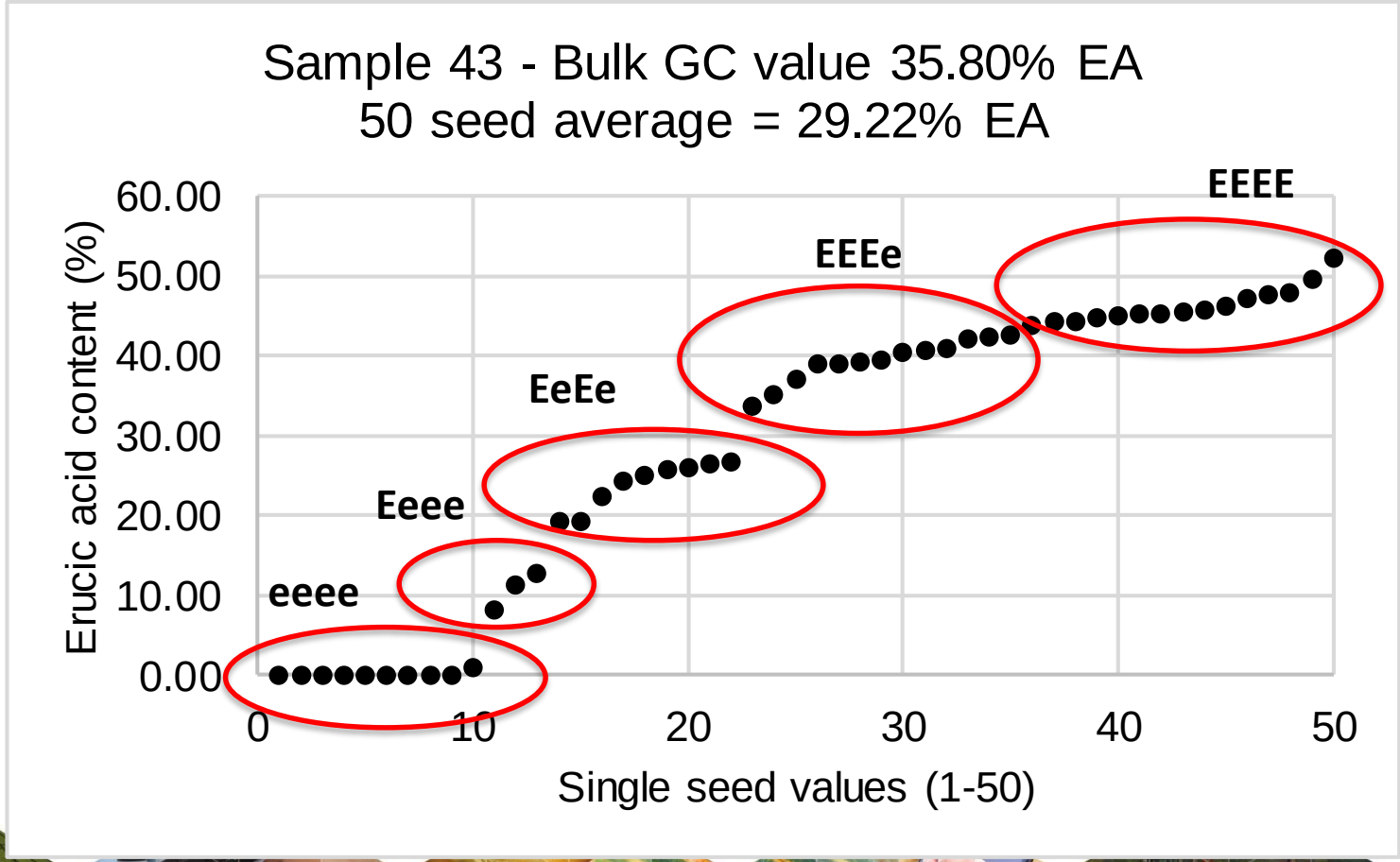

Sample 43
Clean bulk EA = 35.12%; 50 seed EA = 29.22%
Erucic acid content (%)
0
10
20
30
40
50
60
0
10
20
30
40
50
Single seed values (1-50)


Sample 48
Clean bulk EA = 2.11%; 50 seed EA = 1.00%
Erucic acid content (%)
0
10
20
30
40
50
60
0
10
20
30
40
50
Single seed values (1-50)

Our star sample: 35.8 % erucic acid!



Our star sample: 35.8 % erucic acid!



Conclusions from the single seed tests

- Contamination is coming largely from high erucic volunteers, in some cases accumulated and cross-pollinated with sown double-low crops over many rotations.
- The low EA trait does seem environmentally very stable indeed.
- The problem can only be amplified by home saving seed.

Overall conclusions on elevated erucic acid

- NIRS testing is good but not perfect.
- Weed seeds were not an effect here but remain a threat.
- Volunteers, accumulated over many years provided the main effect in these samples.

- We recommend extreme care to avoid volunteers when farm saving seed.
- We recommend the use of Clearfield™ varieties on badly contaminated land, using the associated herbicides to remove volunteers.

Acknowledgement

Grateful thanks to AHDB for their funding and the research opportunity presented by this highly topical and important project, affecting as it does, all links in the oilseed rape supply chain.