THE PRESENCE OF SINAPIS ARVENSIS L. IN RAPESEED

M. ANDREW[1*], R.J. HAMILTON[1*] and J.B. ROSSELL[2*]

Introduction

E.E.C. regulations require that Brassica Napus and Brassica Campestris (rape seed) must not be contaminated with more than 2% of Sinapis Arvensis seed. Since Sinapis Arvensis (wild mustard) is a common weed infestation in European and Canadian rapeseed, an effective and rapid method of determining the level of the contaminant would be invaluable.

At present, the method of differentiating between rape and Sinapis Arvensis depends heavily on visual inspection under a lens or microscope.

The present preliminary communication explores alternative chemical methods to discriminate between rapeseed and this weedseed contaminant.

HPLC of the glucosinolates of rapeseed and other cruciferae species has been studied, first by Helboe and Co-Workers (1). However, the work-up procedure is elaborate and time consuming and whilst the HPLC of the intact glucosinolates eliminates some of the problems associated with enzymatic decomposition, there are a number of overlapping glucosinolates.

Other chemical constituents which might be used as quantitative indicators of wild mustard contamination in rapeseed eg. glucosinalbin and fatty acid composition vary greatly from variety to variety and thus make quantitation difficult.

(1*) Chemistry and Biochemistry Department., Liverpool Polytechnic, Byrom Street, Liverpool L3. England.

(2*) Oils and Fats Section, Leatherhead Food Research Association, Randalls Road, Leatherhead, Surrey. KT22 7RY. England.

Results and Discussion

Early experiments involving the chromatography of intact glucosinolates on silica thin layer plates proved unsuccessful in detecting differences between Sinapis Arvensis and rapeseed, whether normal TLC plates or high performance TLC plates were used. The method according to Croft (2) was used and it was hoped that separation of the glucosinolates would be improved with the relatively new, and previously untried, technique of HPTLC, using a Camag - U - Chamber. However, results were disappointing and, although elution time was reduced to ~ 20 minutes, separation of the glucosinolates was too poor to permit this technique to be adapted for the quantitative assay of glucosinalbin. The individual glucosinolates all appear light blue and separation was minimal - to the extent that the Shimadzu CS - 920 TLC Scanner recorded the total glucosinolates as one spot. The separation might have been improved if additional clean-up steps had been incorporated but the overall procedure was already time consuming.

As an alternative it was decided to examine the surfaces of intact seeds. The surfaces are covered with a wax layer as are all aerial parts of plants (3). In an earlier study one of us (4) had shown that the hydrocarbons isolated from seeds appeared to act as a taxonomic criterion. The normal extraction procedure for surface waxes is to dip the plant material in chloroform for 30 seconds. However, it was felt that such a solvent might leach lipids from the inside of the seed, so hexane for 60 seconds was taken as the extraction solvent.

Several varieties of rape and other members of the cruciferae were extracted and the solvent blown off with nitrogen. The crude lipid extract was redissolved in a known volume of chloroform and then the weight equivalent of 6 seeds was analysed by GLC using a 3% OV 17 packed column, on a GCD Pye-Unicam Gas Chromatograph at 220^{o}C isothermal. At present, no positive identification of any of the peaks has been possible as this work is still in its preliminary

stages, but the peaks range in chain length from 1500 to 3000 Kovats retention indices. The results are shown in table 1.

It is apparent from table 1 that peak no 20 is present in high concentrations in rapeseed both high and low glucosinolate, but in low concentrations in Sinapis Arvensis.

Conclusions

It is hoped that when rapeseed is mixed with Sinapis Arvensis the proportion of peak no 20 can be used to determine the proportion of the weed seed in Brassica Napus and Brassica Campestris, or perhaps, after clean-up procedures, the wax composition of the weed seed will prove substantially different from that of rapeseed, and this criterion may be used as a quantitative indicator of wild mustard in rapeseed.

References

1. P. Helboe, O. Olsen and H. Sorensen, J. Chrom. 1980, 197, 199-205.
2. Anthony G. Croft. J.Sci.Food Agric, 1979, 30, 417-423.
3. B.E. Juniper, Endeavour, 1959, 18, 20-25.
4. S.O. Brown, R.J. Hamilton and S. Shaw, Phytochemistry, 1975, 14, 2726.

TABLE 1

Relative Concentrations (%)

	Sample								
Seed Equivalent Injected.	HG 6	LG 6	EO 6	JN 4	SA 6	FWM 6	B 5	C 6	BS 6
Peak No 1.	0.1	0.1	-	0.1	0.7	0.5	0.1	0.2	-
2.	0.1	0.1	-	0.1	0.4	0.5	0.1	0.2	0.1
3.	-	0.2	-	-	0.9	-	-	-	-
4.	0.8	0.4	-	0.2	1.4	4.6	0.9	1.6	0.4
5.	0.2	0.4	0.1	0.2	0.9	0.5	0.1	0.6	0.3
6.	0.1	0.2	0.1	0.2	0.7	0.6	0.1	0.4	1.7
7.	-	-	-	0.1	-	-	-	-	-
8.	0.3	0.6	0.3	0.1	1.7	2.0	0.2	1.0	0.6
9.	-	-	-	0.1	0.9	-	0.1	0.1	-
10.	0.3	0.7	0.3	0.3	2.1	2.2	0.2	1.1	0.5
11.	0.2	0.5	0.4	0.5	2.1	2.1	0.3	1.3	0.6
12.	6.9	17.0	5.1	5.6	39.2	56.2	4.4	14.5	10.8
13.	0.3	0.4	0.3	0.3	1.4	1.4	0.4	0.6	0.5
14.	-	-	0.6	1.4	-	-	0.1	-	-
15.	1.3	0.9	2.3	2.5	1.7	2.6	1.5	5.0	2.3
16.	-	-	-	-	-	-	-	0.1	-
17.	-	-	0.4	0.8	1.0	-	0.9	0.1	-
18.	3.6	1.5	1.0	0.3	4.3	1.5	1.0	1.8	2.1
19.	-	-	0.3	0.1	3.4	1.3	0.3	0.4	0.5
20.	49.4	49.9	53.8	47.0	7.4	10.0	52.6	11.1	40.9
21.	1.0	2.5	1.2	6.3	10.7	6.6	1.8	5.2	3.2
22.	0.8	2.0	0.8	2.5	1.4	2.6	0.7	-	1.0
23.	-	-	-	-	7.1	-	0.7	0.5	0.5
24.	1.6	1.3	1.7	1.5	3.3	4.9	2.3	18.9	1.5
25.	-	-	-	-	-	-	-	2.7	-
26.	33.0	21.8	32.0	29.5	7.1	-	31.4	-	32.6
27.	-	-	-	-	-	-	-	32.1	-

Table results are the mean of two analyses of two different samples of the same variety.

Key
- HG - low erucic, high glucosinolate Brassica napus, Brassica campestris winter rape.
- LG - low erucic, low glucosinolate Brassica napus, Brassica campestris spring rape.
- EO - Brassica napus, Brassica campestris Elvira, Oxford (rape).
- JN - Brassica napus, Brassica campestris Jet Neuf, Oxford (rape).
- SA - Sinapis Arvensis.
- AWM - Brassica Herta Fine white Mustard.
- B - Brassica oleracea var italica, Broccoli.
- C - Cress, Extra curled.
- BS - Brassica oleracea var gemmifera, Brussel Sprouts.