DEVELOPMENT OF RAPID METHODS FOR THE ESTIMATION OF THE OIL CONTENT OF SINGLE COTTONSEEDS

Part I.—An Oil-Expression Technique

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The rapid estimation of the oil content of a single cottonseed is a matter of considerable importance to studies in cotton breeding relating to the developement of high oil content varieties. If a simple method for this estimation can be evolved, it should be possible quickly to examine a few seeds from an individual plant and use the rest for propagation in breeding experiments. Rapid chemical and electrical methods have been developed by some workers,[1,2,3] and they give good results with several grammes of finely crushed material, but no rapid micro-analytical technique appears to have been developed for work with a single cottonseed. A further elaboration of such a micro method would be to develop a non-destructive oil estimation technique, which does not impair the viability of the seed.

Three methods, each based on a different principle, have been studied in these laboratories: (1) expression of the oil (under pressure) into a set of filter papers followed by estimation of the area of the oil spot, (2) measurement of the density of the oil seed after dehulling, and (3) measurement of the electrical constants of the oil seed under several different conditions. The present paper deals with the first of these methods, which is destructive, while the second and third are both non-destructive, and do not affect the viability of the seed. The results of work on the latter two methods will be communicated in subsequent papers of this series.

Estimation of the Oil Content of Single Seeds by Expression Method

The oil seeds were crushed between several discs of filter paper in order to study the relation between the weight of the seed and the area of the resultant oil spot. The use of several discs of filter paper was adopted to reduce possible error through variable escape of the oil on to the plates of the press. In this study, dehulled seeds from a single variety (L.S.S.) of cotton plant were placed between six discs of Green's No. 401 filter paper, three on either side, and pressed between the plattens of a small 10 ton laboratory bench hydraulic press at various loads ranging from ½ ton to 10 tons. On removal from the press, a well defined nearly circular transluscent patch of oil was visible on the filter papers. The areas of these oil spots on the two innermost filter papers were determined by measuring two mutually perpendicular diameters for each, and the overall mean area was noted, the accuracy being of the order of ±5 sq. mm. in a spot area of 80 to 200 sq. mm. The procedure was repeated six times for each particular load, using six weighed kernels. The results obtained with several loads from 1/2 ton to 10 tons are given in the first four columns of Table 1, and are plotted against the weights of the respective kernels in Fig. 1. The points for each load lie fairly well along the straight line through the origin, the mean scatter about these lines being 5%, corresponding to nearly ±1% in oil content. Thus the areas of the oil spots are closely proportional to the weights of the kernels, from which it can be concluded that, for seeds of a single variety, the ratio of oil content to weight of kernel is nearly constant.

The slopes of the straight lines of Fig. 1, representing the sensitivity, i.e. mean area of the

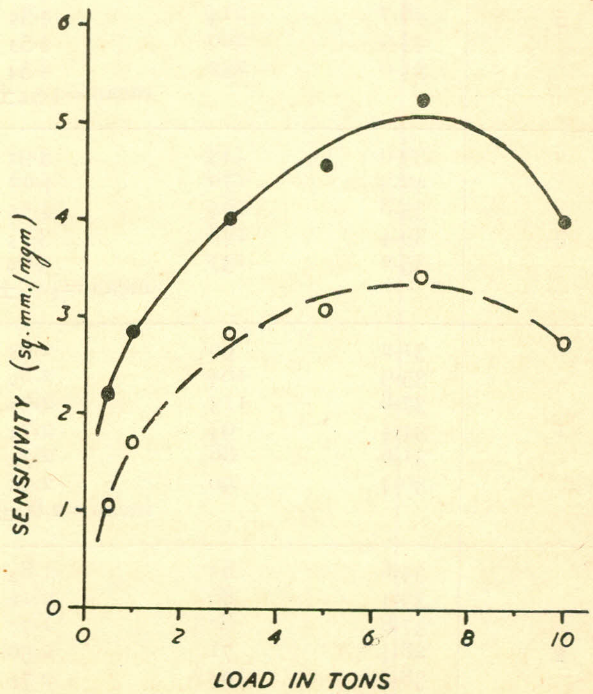

Fig. 2.—Sensitivity curves showing the sensitivity as area of spot per mg. of kernel against the crushing load. Full line for total area of spot, and broken line including correction for area of crushed kernel.

Table 1.—Single Seeds of L.S.S. Variety of Cotton : Expression of Oil between Six Filter Papers (Green's 401).

Total load (tons)	Weight of cotton seed kernel in mg.	Mean total area of oil spot (sq. mm.)	Total area per unit mass (sq. mm./mg.)	Mean area of pressed seed in sq. mm.	Difference (area of spot-area of seed) sq. mm.	Difference per unit mass (sq. mm./mg.)
	46.0	200	4·35	70	130	2.82
	50·4	188	3·73	67	121	2·40
	55·3	206	3·72	63	143	2·58
10	33·8	154	4·55	44	110	3·25
	42.8	153	3·57	53	100	2·33
	37·9	165	4·35	49	116	3·06
			mean=4.04±0.21			mean=2.74±0.17
	45·2	251	5·57	88	163	3·59
	39·7	212	5·34	79	133	3·35
	44·8	232	5·17	86	146	3·25
7	47·2	254	5·38	71	183	3·87
	56·5	284	5·02	95	189	3·34
	56·7	284	5·00	104	180	3·17
			mean=5.25±0.11			mean=3·42±0.11
	44·8	200	4·46	67	133	2·96
	48·5	219	4·51	81	138	2·84
	37·9	188	4·96	59	129	3·40
5	48·7	214	4·39	70	144	2·95
	43·9	200	4·53	59	141	3·21
	51·0	232	4·54	70	162	3·17
			mean=4·56±0.07			mean=3.09±0.10
	36.0	143	3·97	50	93	2·59
	42·3	170	4.02	49	121	2.86
	35·8	164	4·58	47	117	3·26
3	39·4	148	3·75	47	101	2·56
	35·2	137	3·89	35	102	2.89
			mean=4.04±0.12			mean=2.83±0.11
	31·2	90	2.88	44	46	1·47
	35·9	104	2.89	44	60	1.67
	37·8	113	2·97	38	75	1·98
1	34·4	94	2·73	27	67	1·94
	31·6	86	2·72	44	42	1·32
	30·4	90	2·96	35	55	1.80
			mean=2.86±0.05			mean=1·70±0.12
	34·6	63	1.82	33	30	0.86
	39·4	86	2·18	36	50	1·26
	37·8	67	1·77	33	34	0.89
½	28.4	71	2·50	41	30	1.05
	38.2	104	2·72	60	44	1.15
	43·8	95	2·16	44	51	1·16
			mean=2.19±0.15			mean=1.06±0.07

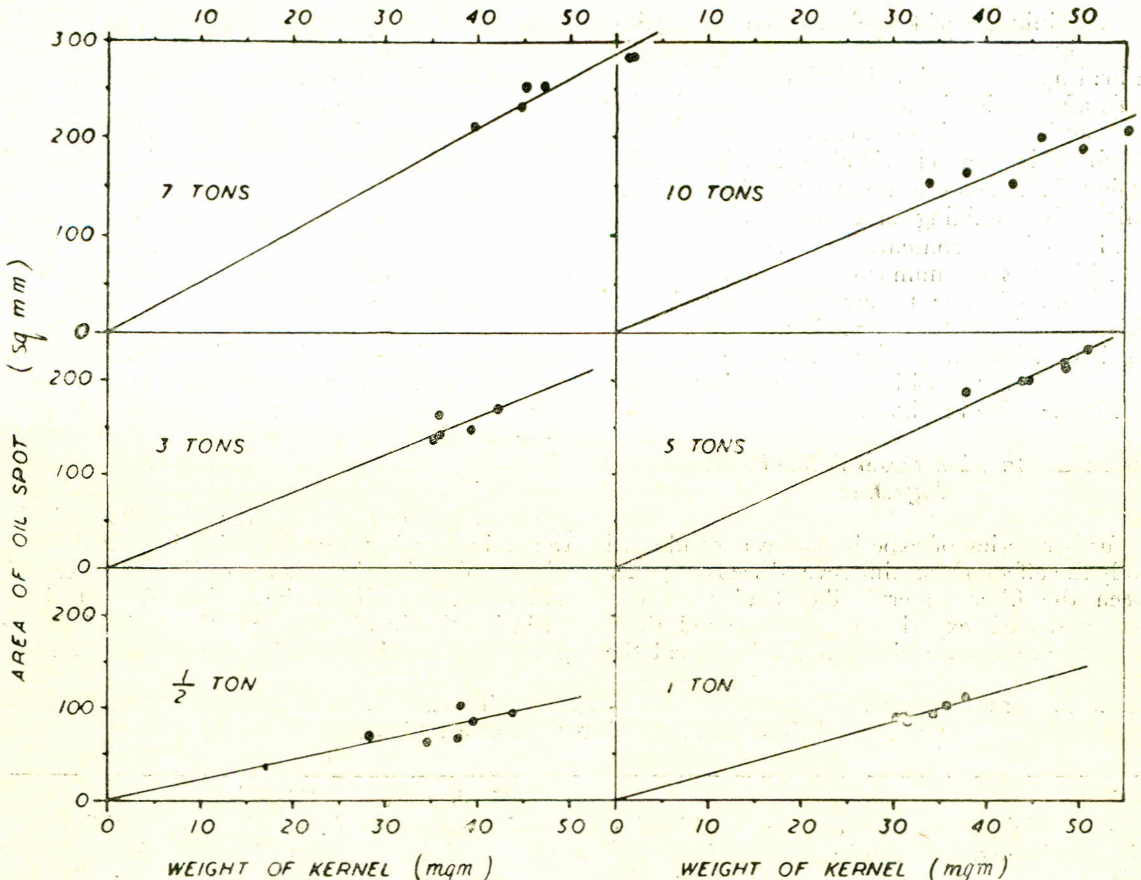

Fig. 1.—Set of graphs for the area of oil spot against weight of L.S.S. cottonseed kernels crushed between "Green's No. 401" filter papers at various loads from 1/2 ton to 10 tons.

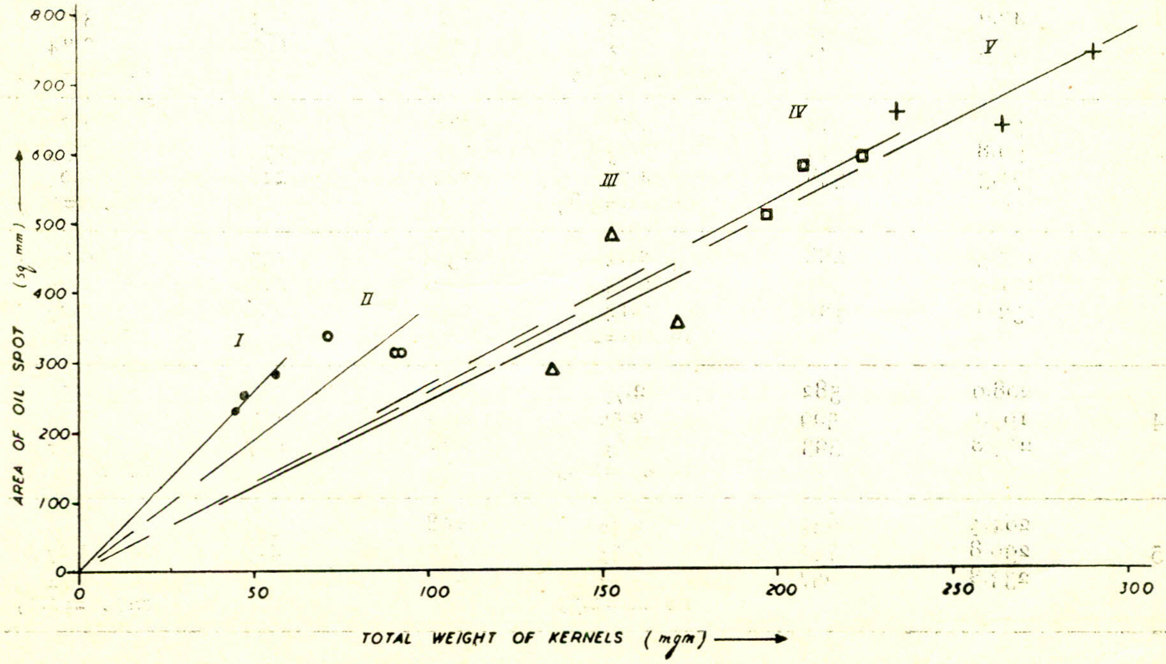

Fig. 3.—Set of graphs for simultaneous crushing of several closely placed seeds, showing area of the spot against total weight of kernels. Solid circles for single seeds; hollow circles for two seeds; triangles for three seeds; squares for four seeds; and crosses for five seeds.

spot per milligram of kernel, are plotted against load in Fig. 2. This sensitivity increases steadily with load upto 7 tons (solid circles and full line in Fig. 2), after which it drops somewhat, indicating that 7 tons is the optimum load in respect of sensitivity for this method of oil estimation. The foregoing data demonstrate the feasibility of this technique for obtaining an accuracy of 1% in the estimation of oil content, provided that loads approaching this optimum value are used. The last three columns of Table 1 show the change in the sensitivity produced by subtracting the area of the crushed kernel from total area of the oil spot, the corresponding sensitivity curve being drawn with a broken line in Fig. 2.

Experiments with Several Seeds Crushed Together

A further series of experiments was conducted to study the effect of crushing several seeds together between the filter papers. The load used was 7 tons, and each experiment was repeated three times. The results are given in Table 2, and the areas of the oil spots are plotted in Fig. 3 against the total weight of the kernels for 1,2,3,4 and 5 seeds. It will be seen from this figure that (a) the sensitivity decreases with increasing number of oil seeds, and (b) the scatter of the points about the lines through the origin is considerably greater for the experiments with multiple seeds than for the single seeds. The first effect is readily understood if we consider the total load of 7 tons to be equally distributed among the seeds being crushed, so that the present experiments actually correspond to loads of 7/2, 7/3, 7/4 and 7/5 tons, respectively, on the individual seeds. This explanation is confirmed by the comparison, shown in Table 3, between the slopes of the lines of Fig. 3 with the sensitivities corresponding to the above loads in Figs. 1 and 2. The steeper drop with increasing number of seeds observed in the experiments with multiple seeds is presumably due to obstruction of the flow of oil by the neighbouring kernels. The increased scatter of the points in Fig. 3 is more difficult to explain, and only a part of it can be attributed to statistical variations in the oil content of the seeds of different plants of the variety used in the present study.

TABLE 2.—SIMULTANEOUS CRUSHING OF SEVERAL SEEDS (FILTER PAPER: GREEN'S 401) SEEDS OF L.S.S. COTTON; TOTAL LOAD = 7 TONS.

No. of cotton-seed kernels	Weight in mg.	Mean total area in sq. mm.	Total area (sq. mm.) per mg. of kernel	Whole area of the pressed seeds in sq. mm.	Difference (area of spot-area of seeds) in sq. mm.	Difference (sq. mm.) per mg. of kernel
	44.8	232	5.17	86	146	3.25
1	47.2	254	5.38	71	183	3.87
	56.5	284	5.02	95	189	3.34
			mean=5.19			mean=3.48
	92.2	314	3.40	188	126	1.36
2	90.8	314	3.45	141	173	1.90
	71.3	338	4.74	122	216	3.02
			mean=3.86			mean=2.07
	135.5	288	2.18	153	135	0.99
3	171.5	354	2.06	132	222	1.29
	153.0	481	3.14	225	256	1.67
			mean=2.46			mean=1.31
	208.0	582	2.79	270	312	1.50
4	197.1	509	2.58	240	269	1.36
	224.8	593	2.63	306	287	1.27
			mean=2.66			mean=1.37
	264.4	637	2.40	298	339	1.28
5	290.8	741	2.55	312	429	1.47
	233.8	657	2.81	253	404	1.72
			mean=2.58			mean=1.49

TABLE 3.—COMPARISON OF ACTUAL AND EXPECTED SENSITIVITIES FOR MULTIPLE SEEDS.

No. of seeds	1	2	3	4	5
Load per seed (tons) ..	7	3.5	2.3	1.8	1.4
Actual sensitivity (sq. mm./mg.)	5.2±0.1	3.9±0.5	2.5±0.3	2.7±0.1	2.6±0.2
Smoothed ,, ,, ,,	5.2±0.1	3.7±0.3	2.9±0.3	2.6±0.2	2.4±0.2
Expected sensitivity (sq. mm./mg.) from Fig. 2	5.1	4.2	3.6	3.3	3.1

Comparison between Results of Extraction and Expression Methods of Estimation of the Oil Content

For this purpose, the measurements on single seeds were also carried out with seeds of another variety of cotton, namely M4, using three different loads near the optimum, viz., 5 tons, 7 tons, and 10 tons, so as to determine the ratio of the oil contents for the M4 and L.S.S. varieties. When the same filter paper and load are used for both varieties, the percentage of oil can be taken as proportional to the area of the oil spots per mg. of kernel. This area per mg. was determined for these three loads, and its ratio to the corresponding quantity for the previously examined variety (L.S.S.) of cottonseed is given in Table 4 for the three loads used. This ratio appears to depend to some extent on the crushing load, with a maximum value at about 7 tons, but the standard errors indicate that this dependance on load is not statistically significant. The overall mean value of the ratio is found in Table 4 to be 0.75±0.04, which is in satisfactory agreement with the ratio 0.71 calculated * from the recorded values[4] of oil content based on the extraction method of estimation on these two varieties as obtained in 1957-58.

TABLE 4.—RATIO OF PERCENTAGE OIL CONTENT FOR M/4 AND L.S.S. VARIETIES.

Crushing load	Ratio from total area of oil spot	Ratio from area of oil spot minus area of crushed kernel	Mean
10 tons	0.83_7	0.70_8	0.77±0 06
7 tons	0.81_6	0.80_7	0.81±0.00
5 tons	0.72_0	0.63_9	0.68±0.04
Mean	$0.79_1 \pm 0.05$	$0.71_8 \pm 0.06$	0.75±0.04

Discussion

The above comparison shows that the indications of oil content given by the expression method can be relied upon to about 1 part in 30, corresponding to nearly ½% oil content, which accuracy compares favourably with that of the extraction methods. The time taken for one determination is of the order of five to ten minutes, which is a great advantage over even the (recently developed) rapid extraction methods ,[1,2] which require from 2 to 8 hours for a single estimation and an average of half an hour per sample if 50 samples are run simultaneously. As against 0.1 g., which is the average weight of the whole seed, samples of 2 to 5 g. are required in the rapid extraction methods for a single estimation of the oil content. The microchemical method described by Iwanoff[5] uses seed fragments (weighing 5-20 mg.), but the oil extraction and estimation techniques are involved and again require several hours.

Two advantages peculiar to the oil expression technique are that (a) it provides a simple means of studying variations within a single variety and even among the seeds from a single plant, of which some indication is given by the scatter of the experimental points about the straight lines in Fig. 1, and (b) the oil spot provides a record of the measurement. This oil spot spreads slowly by diffusion (over a period of several days) at ordinary temperatures, but its boundary is initially very sharp, and it can be traced out with a pencil or developed by

*The agreement would probably be better when allowance is made for the difference between the relative weights of hull and kernel in the two varieties.

spraying with a suitable reagent. Further work is being directed towards refinement of the technique described in this paper and its comparison with the corresponding micro-chemical estimation of oil content of single seeds.

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Note Added in Proof.—For the expression method described above, a constant can be worked out connecting the % oil content (on whole seed basis) with the area of oil spot (in sq. mm./mg. of kernel). For Green's No. 401 filter paper and a crushing load of 7 tons, the foregoing data give a value of 3.6 ± 0.1 for this constant.