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THE EFFECT OF CHEMICAL FERTILIZERS ON THE CONTENT OF OIL, PROTEIN AND GLUCOSINOLATES IN *BRASSICA* INCLUDING RAPESEED

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INTRODUCTION

It is only in recent years that considerable attention has been given to the utilization of fertilizers on rape produced for oil purposes. It is obvious that there are fertilizer requirements for nitrogen, phosphorus and potassium depending on the location of the production and in many cases there are recommendations available for these. However, the requirement for sulfur is not as well defined and because of the high non-amino acid sulfur content of rapeseed it could be very important in the nutrition of the plant. Table I shows the amount of sulfur that is removed from the soil when 1,000 lbs. of rapeseed is harvested. These values do not take into account the other sulfur compounds in the seed or that required by the vegetative portion of the plant. One might estimate that the sulfur requirement is 2 or 3 times this value. It is interesting to note that the requirement indicated in Table I is equivalent to about 15 to 20 lbs. of $(NH_4)_2SO_4$. Radet (reported by Rollier and Ferrif(1)) showed that in the late stages, at the time of flowering and seed ripening, the requirement was estimated to be in the order of 100 kg of SO_3 per ha. (40 kg S per ha).

How does the demand for sulfur compare for different crops? Table II(2) shows that generally hay and cereals have a low requirement; the highest demands are by the high sulfur containing crops, e.g., cabbage and onion. This, as one would expect, also includes rapeseed.

A review of the literature shows that rapeseed is a heavy consumer of nitrogen and phosphorus and very frequently one gets a response in seed yield when these fertilizers are used. In France(4) it is recommended that the following applications be made to winter rape:

TABLE I

THE APPROXIMATE SULFUR CONTENT OF 1,000 LBS. (454 KG) OF RAPESEED

	VARIETY	
	Tanka (*B. napus* L.)	Arlo (*B. campestris* L.)
Glucosinolate	3.3* (1.5)**	2.1 (1.0)
Sulfur amino acids	1.6 (0.7)	1.6 (0.7)

* - Pounds
** - Value in brackets indicates kg.

TABLE II

AMOUNTS OF SULFUR ABSORBED BY CROPS

Crop	Yield/Acre/Year	Sulfur Absorption Pound/Acre
Cereals*	1 ton	9-12
Potatoes	8-9 tons	7-10
Cabbage	13-14tons	19-38
Onions	13-14tons	18-20
Clover Hay	2-3 tons	15-20
Grass Hay	2-3 tons	8-12
Rapeseed**	1 ton	25-35
Barley	1.3 tons	7-14

* - Taken from (2).
** - Taken from work done by J.W. Hamm, Soils Testing Lab., University of Saskatchewan(3).

nitrogen 150 to 200 kilos per hectare, phosphorus 120 to 160 kilos per hectare, potassium 180 to 240 kilos per hectare. In order to obtain maximum benefit from nitrogen fertilizers in winter rapeseed, Rollier(5) suggests that it should be applied at 3 different times during the growing season. In India it was reported(6) that the application of nitrogen increased the uptake of both nitrogen and phosphorus in brown sarson (*Brassica campestris* L.). They also found that an increase in phosphorus increased oil yields whereas an increase in nitrogen did not. In Poland it was observed(7) that a much greater effect was obtained from a spring application than from a fall application on winter rape. In Canada(8) the recommended fertilizer applications for heavy yields are, 0-80 lbs. per acre of nitrogen, 0-20 lbs. per acre of phosphorus and no potassium.

Because of the heavy demands by the *Brassica* species on sulfur, we undertook to investigate the effect of sulfur and other fertilizers on the yield, oil, protein and glucosinolate content on various *Brassica* crops. The investigations were carried out in Saskatchewan at stations located in different soil zones. The results of that study are presented here.

EXPERIMENTAL

The experiments were designed primarily to test the effect of the application of sulfur fertilizers to the grey soils of Saskatchewan. The experiments extended over a four year period and each year the design was slightly modified to gain additional information. Each treatment was replicated four times at each station and for the various assays two replicates were combined to give duplicate values for each treatment.

Four different Brassica crops were grown, yellow mustard (*Brassica hirta* Moench or *Sinapis alba* L.), brown mustard (*Brassica juncea* L. Coss) and two rapeseed crops (*Brassica napus* L. and *Brassica campestris* L.). The stations were selected to represent different soil zones. The pertinent characteristics are summarized in Table III.

The fertilizer treatments are outlined for each year, in 1962 the treatment was two different levels of ammonium sulfate, i.e. 20 and 80, lbs. sulfur per acre (22 and 90 kilos per hectare) and 17.6 and 70.4 lbs. of N/acre. The treatments for 1963, 1964 and 1965 are given in Table IV and V.

The assays for the various seed components were standard methods, i.e. the oil content was determined by the method of Troeng (10).

TABLE III

THE PRINCIPAL SOIL CHARACTERISTICS[a] OF THE FOUR STATIONS REPORTED IN THIS INVESTIGATION

Station	Climatic Zone	Soil Type	Surface Color
Loon Lake	Grey strongly degraded soils	Loam	Light Brownish-Grey
Snowden	Grey and brownish -grey soils	Loam	Grey
Melfort	Deep black soils	Silty clay	Very dark grey
Scott	Dark brown soils	Loam	Dark greyish-brown

a - This information was obtained from the Saskatchewan Soil Survey(9).

TABLE IV

THE FERTILIZER COMPOSITION OF THE VARIOUS TREATMENTS IN THE 1963 EXPERIMENTS

Treatment	COMPOSITION POUNDS/ACRE		
	N	P_2O_5	S
Check	No Additions		
N	70	0	0
P	0	17.4	0
NP	70	17.4	0
NPS_2	70	17.4	80
NS_1	70	0	20
NS_2	70	0	80
N_1S_3	105	0	120

TABLE V

THE FERTILIZER COMPOSITION OF THE VARIOUS TREATMENTS IN THE 1964 AND 1965 EXPERIMENTS

Treatment	COMPOSITION POUNDS/ACRE			
	N	P_2O_5	K	S
Check	No Additions			
N	40	0	0	0
P	0	20	0	0
K	0	0	46	0 (KCl)
NP	40	20	0	0
NPK	40	20	46	0
$NPKS_1$	40	20	46	20 (K_2SO_4)
$NPKS_2$	40	20	46	80 "
$NPKS_3$	40	20	46	120 "

The crude protein content by the Kjeldahl method. The glucosinolate contents of the brown mustard and rapeseed were determined in the earlier experiments by a method described by Wetter(11). Later experiments employed the micro-method described by Youngs and Wetter(12). The glucosinolate content of yellow mustard was determined by a method modified by one of us (LRW) from a method described by Ettlinger and Thompson(13).

The statistics for this study were obtained by carrying out an analysis of variance for a split-plot design(14). The test of significance for a comparison of several means was done by Duncan's multiple range test(15).

RESULTS AND DISCUSSION

OIL CONTENT

The fatty acid composition of rapeseed oil does not appear to be very seriously affected by fertilizers, in other words, adequate supplies are the important factor. In an early investigation by Craig and Wetter(16) it was found that the environment had very little effect on the composition. This suggests that perhaps fertilizers, if adequate, have very little effect on the oil composition. The stations chosen for this experiment(16) were all in the extreme southern part of the four Western Provinces in Canada.

The experiments that are reported here were designed to give a clearer picture of the effect of fertilizers and particularly sulfur on the oil and seed yield of Brassica crops. The experiment was done at one station, Loon Lake, in north-western Saskatchewan in 1962 and the seed yields and percent oil content are shown in Table VI and VII. The data show that sulfur fertilizer treatment significantly increases the seed yield in these crops and decreases the percentage oil in the seed. However the total oil yield per acre is markedly increased and therefore there appears to be a distinct advantage for the application of sulfur fertilizer.

TABLE VI

THE SEED YIELD (LBS. PER ACRE) FOR DIFFERENT BRASSICA SPECIES WITH DIFFERENT SULFUR TREATMENTS IN 1962 EXPERIMENTS

Treatment lbs. S/Acre	SPECIES			
	B. napus	*B. campestris*	*B. juncea*	*B. hirta*
0	1,268	816	1,075	911
20	1,708	850	1,801	1,321
80	1,719	1,109	1,936	1,712

Treatments are highly significant.

TABLE VII

THE PERCENT OIL CONTENT FOR DIFFERENT BRASSICA SPECIES WITH DIFFERENT SULFUR TREATMENT IN 1962 EXPERIMENT

Treatment lbs. S/Acre	SPECIES			
	B. napus	*B. campestris*	*B. juncea*	*B. hirta*
0	49.8	41.9	40.4	31.3
20	48.4	39.8	39.6	31.4
80	46.2	39.4	38.7	29.3

Varieties and treatments are highly significant.

The following year (1963) the experiment was repreated at the same station and the treatment expanded as shown in Table IV. The results for 1963 indicate that there is no significant difference in yield when different treatments are compared (Table VIII). This is different than the experiment done the previous year and it would suggest that conditions vary enough from year to year to discount fertilizer effects on yield. However once again the application of sulfur significantly lowered the oil content of the various *Brassica* species (See Table IX). The application of Duncan's multiple range test at the 5% level showed that the first 3 treatments and the check were significantly higher in oil content than the 4 treatments receiving sulfur.

TABLE VIII

SEED YIELDS FOR THE 1963 EXPERIMENT

	SPECIES			
Treatment	*B. napus*	*B. campestris*	*B. juncea*	*B. hirta*
Check	1,498	1,027	1,366	854
N	1,502	936	1,178	835
P	1,435	1,099	1,310	974
NP	1,615	1,303	1,384	910
NPS_2	1,440	1,130	1,306	1,054
NS_1	1,610	1,054	1,406	996
NS_2	1,536	1,070	1,310	929
N_1S_3	1,272	1,176	1,354	821

Treatments are not significant.
Species are highly significant.

In 1964 the experiment was expanded to another station in north-eastern Saskatchewan. The fertilizer treatment is described in Table V and differs from the previous year in that potassium has been added. The yields, given in Table X, for Snowden were significantly different, although the pattern is not very clear. In *B. campestris* the check is significantly different from the $NPKS_3$ fertilizer treatment; the same is true for the yellow mustard. In neither case were the sulfur treatments different than the NPK treatment. Fertilizer treatments for *B. napus* and *B. juncea* were not significant at the 5% level. The seed yields for Loon Lake are not shown because the crop was very poor in the area that year.

TABLE IX

PERCENT OIL FOR THE 1963 EXPERIMENT

Treatment	SPECIES			
	B. napus	*B. campestris*	*B. juncea*	*B. hirta*
Check	48.1	43.2	41.8	32.0
N	47.2	43.0	40.7	32.3
P	47.3	43.7	41.1	32.4
NP	47.1	42.4	41.4	31.4
NPS_2	44.1	41.2	40.3	29.9
NS_1	44.8	41.9	39.7	30.1
NS_2	46.0	40.4	39.0	29.6
N_1S_3	43.7	40.6	38.3	29.5

Treatments and species are highly significant.

TABLE X

SEED YIELD FOR THE 1964 EXPERIMENT AT SNOWDEN

Treatment	SPECIES			
	B. napus	*B. campestris*	*B. juncea*	*B. hirta*
Check	1,241	1,518	1,534	1,008
N	1,451	1,580	1,641	968
P	1,376	1,767	1,856	1,232
NP	1,497	1,892	1,839	1,238
K	1,247	1,607	1,738	1,168
NPK	1,559	1,722	1,736	1,328
$NPKS_1$	1,404	1,583	1,999	1,595
$NPKS_2$	1,421	1,861	1,866	1,764
$NPKS_3$	1,459	2,063	2,011	1,614

Treatments and species are highly significant.

The oil content was highly significant for both stations as shown in Table XI. These results are shown as the averages of the four species taken at each station. At Loon Lake the highest sulfur application resulted in a significantly lower oil content than the check. The same indication was evident for Snowden. The results once again indicate that the use of sulfur fertilizers tends to decrease the oil content of the seed.

TABLE XI

THE AVERAGE OIL CONTENT OF THE FOUR BRASSICA SPECIES GROWN AT LOON LAKE AND SNOWDEN IN THE 1964 EXPERIMENT

Treatment	STATION	
	Loon Lake	Snowden
Check	40.3	39.2
N	38.1	39.1
P	39.0	39.9
NP	39.5	39.3
K	38.8	39.1
NPK	39.0	39.7
$NPKS_1$	38.7	38.3
$NPKS_2$	37.7	38.1
$NPKS_3$	38.1	37.8

Treatments and Species were highly significant at both stations.

The 1965 fertilizer test was expanded once again to include two more stations, Melfort and Scott. The fertilizer treatments were the same as for the 1964 experiment. A summary of the yield data and an analysis of variance are given in Table XII. The yields from the typical grey soils responded in a highly significant fashion to fertilizer treatment. The black and dark brown soils do not respond to the application of sulfur. This observations was not unexpected as these latter types of soils are not likely to manifest a sulfur deficiency as readily as the typical grey soils. The oil content was highly significant at only one station, Snowden (See Table XIII), and here, as in previous results, the oil content was significantly lower in the sulfur treated plots. The plots receiving treatments $NPKS_2$ and $NPKS_3$ were significantly lower than the check, N, P, or NPK. The only other station which showed any significance was Scott. There is no explanation as to why the other two stations should not have followed the pattern this particular year.

Field trials carried out in Poland, which involved the spring and fall applications of nitrogen, showed that an increase in nitrogen did not affect the percent oil content of the seed but it did affect the oil yield(7). In other words an increase in nitrogen did result in a higher oil yield per hectare. Bachmann(17) noted that the application of 80 kg/ha of nitrogen in different forms had no effect on the oil content of rapeseed or on the fatty acid composition. Our investigations certainly agree with these conclusions.

TABLE XII

THE AVERAGE YIELD FOR THE FOUR *BRASSICA* SPECIES AT FOUR STATIONS IN THE 1965 EXPERIMENT

Treatment	STATIONS			
	Loon Lake	Snowden	Melfort	Scott
Check	1,416	1,412	1,575	1,150
N	1,366	1,455	1,362	1,050
P	1,544	1,614	1,618	1,271
NP	1,548	1,715	1,436	1,142
K	1,579	1,424	1,513	1,033
NPK	1,866	1,575	1,533	1,016
$NPKS_1$	1,843	1,758	1,424	1,118
$NPKS_2$	1,917	1,862	1,308	1,041
$NPKS_3$	1,866	1,789	1,676	1,067
Treatment	HS	HS	NS	NS
Crops	HS	S	HS	NS

HS - highly significant (to the 1% level)
S - significant (to the 5% level)
NS - not significant.

TABLE XIII

THE AVERAGE PERCENT OIL CONTENT FOR THE FOUR BRASSICA SPECIES AT FOUR STATIONS IN THE 1965 EXPERIMENT

Treatment	STATIONS			
	Loon Lake	Snowden	Melfort	Scott
Check	39.5	39.7	38.1	34.0
N	38.9	39.3	37.9	34.0
P	39.6	39.8	37.5	34.0
NP	39.6	39.0	37.5	33.6
K	39.7	38.9	38.0	33.9
NPK	39.1	39.2	37.6	33.6
$NPKS_1$	38.5	39.0	36.9	33.8
$NPKS_2$	39.1	37.9	37.2	33.7
$NPKS_3$	38.9	37.7	37.4	33.2
Treatment	NS	HS	NS	S
Species	HS	HS	HS	HS

Recently a detailed study was carried out on the effect of nitrogen, phosphorus, potassium and sulfur on the content and the composition of oil in Brassica napus L. by Appelqvist(18). He found that phosphorus and potassium had no effect on oil content, but high nitrogen tended to decrease it and lack of sulfur indicated the same thing. In zero sulfur the oil yield was low but a small addition of sulfur doubled the oil yield with no further increase with increased sulfur. This infers that the genetic make-up for oil composition is basic and is not seriously interfered with by fertilizer treatment. Nitrogen levels did have some effect on the composition of rapeseed oil particularly the palmitic, eicosenoic and erucic acid contents, however phosphorus and potassium and combinations of these and nitrogen had no effect. Sulfur fertilizer appears to affect the fatty acid composition, e.g. higher percentages of oleic and eicosenoic and lower percentages of linolenic and erucic.

PROTEIN CONTENT

The nitrogen content of rapeseed usually increased in response to the addition of fertilizers. The importance of nitrogen fertilizers is self-evident and in many reports the results are quite spectacular. Once again the work of Horodyski(7) shows that the quantity of nitrogen in the seed increases very markedly (about 100%) when nitrogen fertilizers are added to Polish crops. The same thing is observed in the rape straw. There appears to have been very little investigation on the effect of sulfur fertilizers on the nitrogen content of rapeseed. Josefsson and Appelqvist(19) in laboratory experiments in plastic boxes showed that the nitrogen content of rapeseed increased with the addition of small amounts of sulfur but further additions gave no additional response. In another report Josefsson(20) found that there was a positive correlation between nitrogen fertilizer and nitrogen content in the seed but sulfur did not exert any effect. This worker also did amino acid analyses and the fertilizer levels used indicated very little effect on the amino acid content, however, when the amount of sulfur was severely restricted then the sulfur amino acids decreased.

The results for the simple 1962 experiment are summarized in Table XIV. The addition of sulfur did not affect the protein content (N x 6.25) of the different Brassica species. The data in Table XV shows that the results for the 1963 experiment are very similar to the previous year's results. The treatment was highly significant and further statistical analysis demonstrates that the difference is in the two rapeseed varieties. Further inspection showed that the addition of sulfur to the nitrogen treatment did not increase the protein content. However, addition of nitrogen and sulfur did give a significant increase over the check or addition of phosphorus.

The protein content for the 1964 experiment is presented in summary in Table XVI. The difference in protein values in Table XVI as compared to previous tables are due to the base employed. In the 1962 and 1963 experiments the percent protein was based on oil-free seed, in the 1964 and 1965 experiments it was based on the whole seed. The application of different combinations of fertilizer has no significant effect on the yield of protein. The species differ significantly from one another with yellow and brown mustard having a somewhat higher protein content than rapeseed.

TABLE XIV

THE PROTEIN CONTENT OF *BRASSICA* SPECIES WITH VARIOUS SULFUR TREATMENTS IN 1962 EXPERIMENT

Treatment lbs. S/Acre	SPECIES			
	B. napus	*B. campestris*	*B. juncea*	*B. hirta*
0	40.3	40.5	41.4	46.2
20	40.6	41.6	41.6	45.0
80	41.1	41.4	41.5	43.7

TABLE XV

THE PROTEIN CONTENT OF *BRASSICA* SPECIES GIVEN DIFFERENT FERTILIZER TREATMENTS IN THE 1963 EXPERIMENT

Treatment	SPECIES			
	B. napus	*B. campestris*	*B. juncea*	*B. hirta*
Check	44.0	42.7	46.4	48.6
N	45.2	44.0	46.7	49.5
P	43.7	42.3	47.1	48.2
NP	46.0	44.6	47.2	50.5
NPS_2	46.4	45.5	47.0	49.9
NS_1	46.5	45.0	46.6	50.5
NS_2	46.3	46.0	48.2	49.9
NS_3	47.6	46.2	48.9	50.5

Both treatments and varieties were highly significant.

TABLE XVI

THE PROTEIN CONTENT (N x 6.25) FOR FOUR BRASSICA SPECIES GROWN AT TWO STATIONS AT DIFFERENT LEVELS OF FERTILIZER FOR THE 1964 EXPERIMENT

Treatment	STATION	
	Loon Lake*	Snowden*
Check	23.6	25.0
N	24.7	25.2
P	23.5	24.3
NP	23.8	24.8
K	23.6	25.0
NPK	24.2	25.5
$NPKS_1$	23.9	25.4
$NPKS_2$	24.0	25.4
$NPKS_3$	24.4	26.4
Treatment	NS	NS
Species	HS	HS

* - Average value obtained for the four *Brassica* species at that station.

The results of the 1965 experiment are summarized in Table XVII and they show once again that the fertilizer treatments have a highly significant effect on the protein content. However for the first time we have a station (Snowden) that shows that the application of sulfur does significantly increase the protein content over its check (in this case NPK). It is interesting to note that this was not the case at this station the previous year. The other stations, Scott and Loon Lake, showed a different result and that was that sulfur did not give higher protein contents than the check plot (NPK). It is perhaps significant that the sulfur response occurred on the grey soils where one would expect to find a low sulfur content in the soils.

The use of fertilizers does not appear to have too great an effect on the protein content of rapeseed, however there does appear to be a beneficial effect when sulfur is used on our Canadian grey soils. Work carried out by Josefsson and Appelqvist(19, 20) suggest that the effect is small but that the sulfur amino acids in experiments where sulfur supplies are low are also low. Recent work done by Finlayson and co-workers(21, 22) indicate that there can be significant differences in protein fractions isolated from rapeseed grown in sulfur deficient soils. They have shown that the yield of

TABLE XVII

THE AVERAGE PROTEIN CONTENT (N x 6.25) OF FOUR BRASSICA SPECIES GROWN AT FOUR DIFFERENT STATIONS AT DIFFERENT FERTILIZER LEVELS IN THE 1965 EXPERIMENT

	STATIONS			
Treatment	Loon Lake	Snowden	Melfort	Scott
Check	27.4	27.2	28.6	31.9
N	28.3	27.3	28.5	32.1
P	27.1	27.0	28.6	31.4
NP	27.8	27.2	28.6	32.5
K	27.4	26.9	28.4	31.7
NPK	27.6	26.9	28.5	32.3
$NPKS_1$	28.6	27.7	28.6	32.3
$NPKS_2$	28.4	28.6	28.6	31.9
$NPKS_3$	28.3	28.4	28.8	32.3
Treatment	HS	HS	S	HS
Species	HS	HS	HS	HS

two protein fractions S_{12} (BI) and S_2 (AIVS) are greatly increased by the use of sulfur fertilizer on grey soils(21). In addition they have shown that the sulfur amino acids increase somewhat with sulfur fertilization which agrees with the Swedish workers. In another report Finlayson and co-workers(22) show that the crude protein value obtained from a Kjeldahl nitrogen analysis indicates that it is not an accurate measurement of protein. Table XVIII taken from their paper shows this very nicely, note particularly the tremendous differences in the S_{12} and S_2 fractions isolated from the rapeseed meal receiving various kinds of fertilizer treatment.

GLUCOSINOLATE CONTENT

There has been considerably more investigation done on the effect of sulfur nutrition on the glucosinolate content than on either nitrogen or oil content. This interest undoubtedly arises from the fact that these compounds in part are responsible for the adverse effect of rapeseed meal when fed to animals and poultry. Since glucosinolates contain a high percentage of sulfur (approx. 15%), one would expect that the sulfur nutrition of the plant would have a marked effect on the glucosinolate content of *Brassica*. In 1959 Craig and Wetter(23) reported differences in glucosinolate content depending on where the rapeseed was grown.

TABLE XVIII

NITROGEN RECOVERIES FROM THE AQUEOUS RAPESEED EXTRACTS (MG N/GM OF MEAL N)[*]

Fraction	Wt. of Nitrogen Recovered (mg)			
	Check	N	NP	$NPKS_2$
Meal Nitrogen	905	936	950	952
Buffer-Soluble Nitrogen	560±20	560±25	502±25	540±30
TCA-Precipitated Nitrogen	354± 5	366± 5	367± 5	396±10
TCA-Soluble Nitrogen	62±13	97±15	102± 5	10± 1
12S Globulin N	165.0	170.0	200.0	210.0
AIVS Protein N	32.0	37.0	50.0	155.0
Free Amino Acid Nitrogen	2.6	5.8	6.2	3.3
Nucleic Acid Nitrogen	45.0	52.0	50.0	45.0

* - This table is taken from (22).

The experimental results for the 1962 experiment are presented in Table XIX. They have not been treated statistically but there is no doubt that the application of sulfur fertilizers to the soil at Loon Lake produced an increased glucosinolate content in each of the Brassica species tested.

TABLE XIX

THE TOTAL MUSTARD OIL CONTENT OF THE DIFFERENT BRASSICA SPECIES GROWN AT DIFFERENT LEVELS OF SULFUR IN THE 1962 EXPERIMENT

Treatment lbs. S/Acre	SPECIES			
	B. napus	B. campestris	B. juncea	B. hirta
0	3.06[*]	4.53[*]	3.93[**]	13.8[***]
20	5.79	7.24	8.83	21.7
80	7.36	7.61	9.87	19.5

* - Value is the sum of isothiocyanate expressed as mg 3-butenyl isothiocyanate and mg of oxazolidinethione per gm of oil-free meal.

** - Value is expressed as mg allyl isothiocyanate per gm of oil-free meal.

*** - Value is expressed as mg p-hydroxybenzyl isothiocyanate per gm of oil-free meal.

The data presented in Table XX illustrates that the application of sulfur fertilizer causes an increase in the glucosinolate content of Brassica. There was no difference when the check was compared to nitrogen, phosphorus or a combination of the two, but when sulfur was added all levels were significantly higher than those receiving no sulfur. The results for B. hirta indicate the same thing, i.e. the addition of sulfur at Loon Lake results in significantly higher glucosinolate content.

TABLE XX

THE AVERAGE MUSTARD OIL CONTENT[☆] OF THREE BRASSICA SPECIES[☆☆] RECEIVING DIFFERENT FERTILIZER TREATMENTS AT LOON LAKE IN 1963 EXPERIMENT

Treatment	Mustard Oil Content[☆]
Check	4.51
N	4.25
P	5.79
NP	4.14
NS_1	7.80
NS_2	9.04
NS_3	9.43
NPS_2	9.06

Varieties - not significant.
Treatment - highly significant.

☆ - This is an average value derived from the four major glucosinolates found in the three Brassica studied.

☆☆ - The three species were B. napus, B. campestris, B. juncea.

The average mustard oil content for the 1964 experiment are presented in Table XXI. Since we were primarily interested in the effect that fertilizer treatment had on mustard oil content of Brassica, we average all varieties for a single treatment. It is evident that the application of sulfur increased the mustard oil content of Brassica grown on these grey soils. The treatment is highly significant. These data verify the results that were obtained the previous year at Loon Lake. A look at the data for each Brassica species shows that the only species that responded significantly to sulfur treatment was B. hirta. This is rather difficult to explain but one probable explanation is the tremendous variation in the replicate samples thus affecting the error. In other words, at times variation between replicates was greater than between treatments. We feel that this effect could have something to do with availability of sulfur on those plots not receiving sulfur as here the variability was the greatest.

TABLE XXI

MUSTARD OIL CONTENT[a] OF FOUR BRASSICA SPECIES GROWN AT TWO STATIONS WITH VARIOUS FERTILIZER TREATMENTS IN 1964 EXPERIMENT

Treatment	STATION	
	Loon Lake	Snowden
Check	10.19	9.95
N	10.54	8.83
P	11.46	9.89
NP	9.73	10.14
K	11.28	8.53
NPK	8.89	9.09
$NPKS_1$	12.81	12.83
$NPKS_2$	13.28	13.09
$NPKS_3$	13.38	14.18
Varieties	HS	S
Treatments	HS	HS

[a] - These are average values for five different types of mustard oil found. See footnote in Table XIX for details.

Table XXII presents the data for yellow mustard alone and it shows that the treatment was not significant at Loon Lake and just significant at Snowden. Why this should be so different than the average of the four species is not known at this time, except perhaps the variability in replication.

The results of the 1965 experiment are summarized in Table XXIII. Once again treatment resulted in highly significant effects in the glucosinolate content. In all cases there did not appear to be any effect of treatment on the two rapeseed plantings or on *B. juncea*. Sulfur treatment caused a significant increase in glucosinolate content of the yellow mustard at three stations, Loon Lake, Snowden and Melfort. The significant increase is compared with the NPK treatment. It was observed that at Scott this was not so, therefore verifying once again that sulfur treatment has an effect on grey soils but it appears to have no effect on dark brown soils which presumably are already high in sulfate.

TABLE XXII

THE ISOTHIOCYANATE CONTENT OF B. HIRTA AT TWO STATIONS WHEN DIFFERENT LEVELS OF FERTILIZER WERE APPLIED IN THE 1964 EXPERIMENT

Treatment	STATION	
	Loon Lake	Snowden
Check	20.3	21.1
N	21.8	18.5
P	24.3	21.0
NP	20.2	21.4
K	23.6	17.4
NPK	18.7	18.9
NPKS1	26.2	26.3
NPKS2	27.0	26.3
NPKS3	27.5	31.0
Treatment	NS	S

TABLE XXIII

THE AVERAGE MUSTARD OIL CONTENT[a] OF FOUR BRASSICA SPECIES GROWN AT FOUR STATIONS WITH VARIOUS FERTILIZER TREATMENTS IN 1965 EXPERIMENT

Treatment	STATIONS			
	Loon Lake	Snowden	Melfort	Scott
Check	12.64	8.28	14.21	14.28
N	11.20	7.95	13.91	12.86
P	10.34	7.86	12.09	14.53
NP	12.33	8.16	12.30	13.68
K	12.06	7.64	14.29	12.99
NPK	11.65	8.00	11.20	14.56
NPKS1	13.20	11.10	13.73	13.80
NPKS2	14.76	12.96	13.28	14.36
NPKS3	15.11	12.96	13.13	14.34
Species	HS	HS	HS	HS
Treatment	HS	HS	HS	HS

a - These are average values for the five different mustard oils assayed. See footnote in Table XIX for details.

A review of the literature indicates that other workers have in general made the same observations. Trzebny's(24) studies on winter rape varieties in Poland found that high rates of nitrogen fertilizer resulted in a decreased content of glucosinolates. Our results would tend to substantiate this observation. Josefsson and Appelqvist(19) and Josefsson(20, 25) have found that there is a positive correlation between glucosinolate content and sulfur content of the soil or growth medium. Some of Josefsson's work(20) verified Trzebny's conclusion regarding the use of high nitrogen fertilization. He(20) also showed that low sulfate nutrition had a far greater effect on glucosinolate content than on the sulfur amino acids. In general other workers(26) have found the same general relationship between glucosinolate and sulfur fertilization.

CONCLUSION

The results of the fertilizer experiments carried out over a period of four years may be summarized as follows:

1. In general the seed yield gave a positive response to the application of fertilizers. This was particularly true for the application of sulfur fertilizers to the grey soils.

2. The percent oil decreased with the application of sulfur fertilizers, however the total yield of oil per acre increased very significantly.

3. The effect on the protein content was variable. In some years the response to fertilizers was considerable, e.g. an increase in crude protein was obtained when sulfur fertilizers were utilized. Again this was particularly true for the grey soils.

4. The effect on the glucosinolate content was very noticeable, i.e. the application of sulfur fertilizers resulted in a significant increase in the glucosinolate content. Again the effect was most pronounced on the grey soils.

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