THE NITROGEN-SULPHUR INTERACTION IN LENTILS

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The lentil is one of man's oldest food crops and provides a valuable protein source. This, coupled with its ability to thrive on poor soils and under adverse conditions ensured its survival as a crop species. Nevertheless, twenty years ago the crop was unknown in western Canada. Since then the area sown to lentils has steadily increased to 105,000 hectares in 1986 (Sask. Agric. 1986).

Lentils are now being grown not only as a pulse crop, but also as a green manure crop to help reverse soil degradation by increasing soil organic matter and soil nitrogen. As a result, lentils are increasingly being grown on soils that are deficient in S or are potentially deficient in S. In addition, lentils may also be grown on soils that are very high in available S such as areas tending towards salinity. However, very little is known about the S nutrition of lentils, the levels of S in the plant, and the effects oflow or high levels of available N and/or S upon those S levels.

Sulphur, like N, is primarily a constituent element of plant protein. Consequently, a deficiency of S will limit protein synthesis and result in the accumulation of non-protein N (Stewart and Porter, 1969). Conversely, N deficiencies will restrict the incorporation of S into plant protein and result in accumulations of non-protein S. Thus, N and S are closely linked in plant nutrition and the efficiency of the assimilation of one element is directly related to the comparative availability of the other element in the soil medium.

Numerous attempts have been made to determine the S status of the plant by tissue analysis. Methods based upon a single S analysis have generally proved unsatisfactory. A more reliable basis for diagnosis, due to the inter-relationship noted above, is provided by the determination of the N:S ratio in the plant. Nevertheless, for legumes this ratio can vary widely. Nuttal(1985) reported 14 to 21:1 for alfalfa hay. An alternative diagnostic test relies on the decline in inorganic S in the plant tissues below a species-specific threshold (170-330 ppm; Dijkshoorn, W. and van Wijk, A.L. 1967)

The studies herein were set up to examine the S nutrition of lentils as affected by N and to determine diagnostic indices of S deficiency in lentils.

MATERIALS AND METHODS

Field Studies

Field trials were carried out at Melfort and at Loon Lake in the summer of 1985. At Melfort the plots were laid out on a Waitville loam soil well supplied with available N and S (Table 1). At Loon Lake the soil was a Loon River loam soil with low levels of available N and S (Table 1).

Two cultivars, Eston (a standard grain legume cultivar) and Indianhead (a new cultivar developed for use as green manure) were seeded into plots of eight rows (Melfor or four rows (Loon Lake), each row 6m long and 23cm from the adja ent row. Seeding rates were equivalent to 35kg ha^{-1} and 25kg a $^{-1}$ for Eston and Indianhead respectively.

At both locations N was applied as urea in a side-band at rates of 0, 20, 40, and 80 kgN/ha and S as K_2SO_4 at rates of 0,10,20,and 40 kgS ha^{-1} in a 4x4 factorial. Seeds we e inoculated with <u>Rhizobium leguminosarum</u> prior to seeding. The t eatments were replicated 4 times and arranged in a split plot desi n with cultivars as the main plot and the fertilizer treatm ts randomized within each cultivar. Blanket applications of K and were applied.

Plots were sampled three times during the owing season: after approx 50 days (vegetative), 76 days (flowerin , and 114 days (harvest). The first two samplings consisted of etre-row samples. Harvest samples at maturity consisted of on whole row at Loon Lake and two whole rows at Melfort.

Plant samples were dried at 60C and weighe Harvest samples were thrashed, the grain separated, cleaned, eighed and ground in Wiley mill. Samples of the straw, chaff, i ature seeds, and other screenings following seed cleaning were al retained, mixed and ground together in a Wiley mill. Plant sam es, straw, and seed samples were analyzed for total N, total P, otal K, total S, and extractable SO_4-S.

The plant tissue samples were analyzed for otal N, total S, and water-extractable SO_4-S. Total P and total K ere also determined but are not reported herein.

All yield, nutrient uptake, and plant comp sition data were analyzed as a 4x4 factorial design. All 4 repli ates were used for the Melfort data but at Loon Lake, heavy floodin had severely damaged a substantial portion of one replicate. The ta for this replicate was removed and analysis carried out using 3 replicates.

<u>Growth Room Study</u>

A Sylvania fine sandy loam soil, a member the Grey Luvisols, with 5 µg NO_3-N/g and 2 µg SO_4-S/g availab e and a total S of 125 µg S/g was used in this study. The soil had ot grown legumes within the last 15 years. Samples of the sur ace horizon were collected, air-dried, passed through a 2mm siev and thoroughly mixed to ensure uniformity.

Sealed pots containing 1000g air-dried soi mixed with 500g washed quartz sand were seeded with inoculated eed of the black-seeded lentil variety "Indianhead". Nitrogen, s a solution of NH_4NO_3, was applied by injection to depth of 2.5 m at rates of 0, 33, 100, and 300 µg N/g soil. Sulphur, as a solut on of K_2SO_4, was also applied by injection at rates of 0, 5, 15, d 45 µg S/g soil to the inoculated lentil pots. All possible com inations of the N and S levels were represented and replicated s x times. Sufficient rates of other macro- and micro-nutrients were added to ensure that elements other than N and S were not lim ting.

Location	Depth (cm)	Texture	pH	NO3-N (ppm)	SO4-S (ppm)	Conductivity (S m^{-1})
MELFORT	0-15	Loam	7.2	26.0	12	.65
	15-30	loam	7.5	7.5	7	.5
	30-45	loam	7.7	5.0	12	.6
	45-60	clay loam	7.8	3.5	>12	1.0
	60-90	clay loam	7.8	1.75	>12	1.7
LOON LAKE	0-15	loam	7.0	6.0	4.5	.2
	15-30	loam	6.7	4.0	4.5	.2
	30-45	clay loam	6.4	2.0	3.5	.2
	45-60	clay loam	6.4	1.75	3.5	.2
	60-90	clay loam	6.4	1.5	3.0	.2

TABLE 1: Some chemical properties of the soils at Melfort and Loon Lake used in the study of N and S fertilizer effects in the field.

For comparison, another two pots/rep were seeded with 'Westar' rapeseed. One pot was treated with 300 μgN/g soil and the second with 300 μgN/g soil plus 45 μgS/g soil.

Following emergence, the lentil plants were thinned to three plants per pot and grown in a growth chamber with a 16hr day(24C) - 8hr night(12C) cycle. On the 72nd day after seeding, when most of the lentils were in full bloom, three replications were harvested. The remaining pots were allowed to grow to maturity and then harvested. As far as possible, leaves which absized during the plant growth were collected and incorporated with the harvested tissue. All plant samples were dried at 80C, weighed, and in the case of the mature plant samples, seed was separated and weighed.

Plant samples were finely ground in a coffee mill and analyzed for total N, total S, NO_3-N, HI-S, SO_4-S. Isotopic analysis was also carried out.

Analyses of variance were calculated for each sampling period separately on all yield, nutrient uptake, and plant composition data (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Field Studies

The pattern of response by the two varieties of lentils to the various N and S treatments was similar at both locations. At Melfort, the total dry matter yield of Eston at flowering was significantly greater than the dry matter yield of Indianhead (Fig.1). However, Indianhead is a later maturing and more indeterminate variety than Eston. Since both varieties were cut on the same day, Indianhead would have been physiologically less mature than Eston hence the lower weight. Even at maturity, when both varieties were harvested, Indianhead was greener and this suggested that with a longer growing season, Indianhead would have had a higher dry matter yield.

Applications of N and S had little effect upon yield as the soil at Melfort was well supplied with both N and S. There was

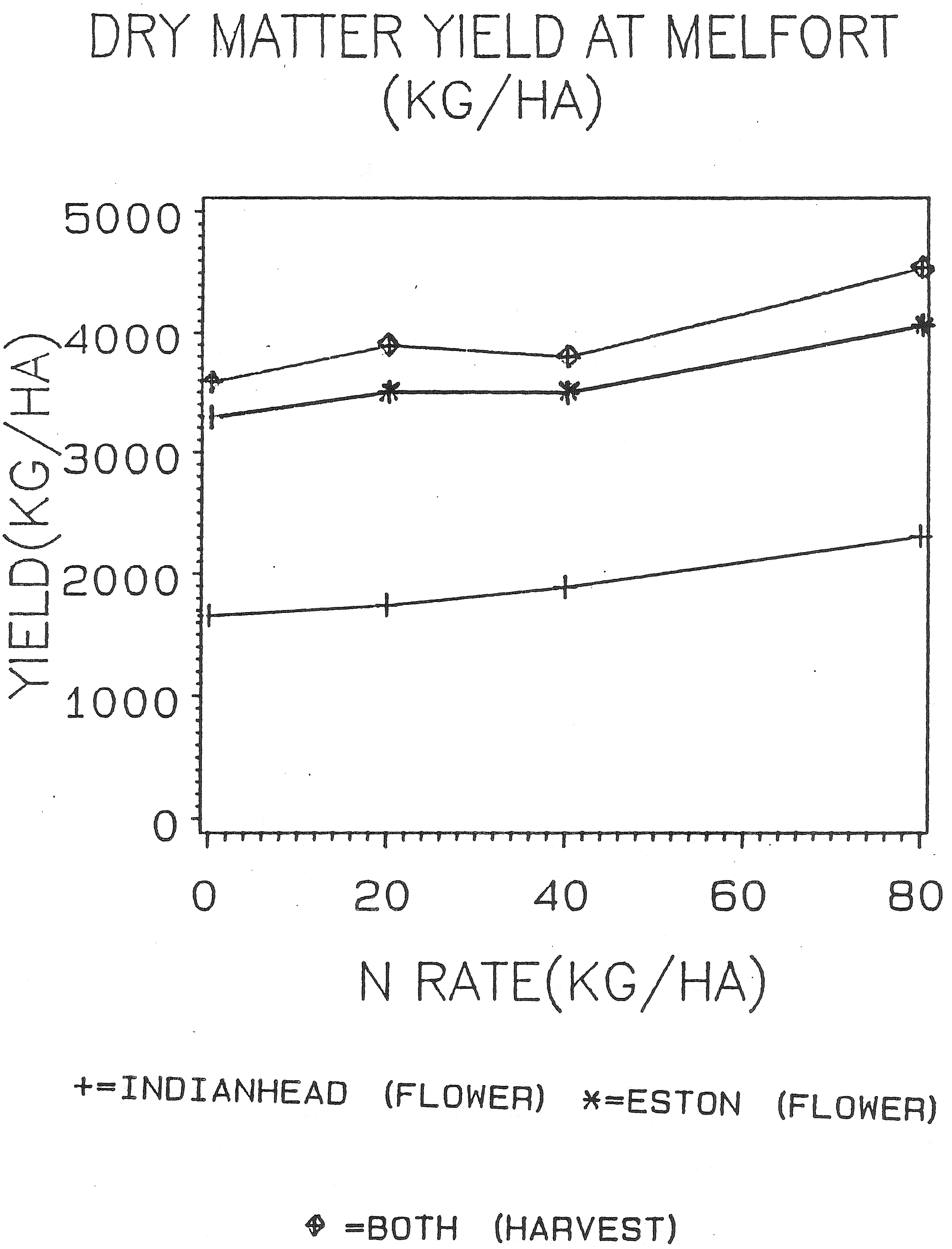

Fig 1: Effect of N applications on the dry matter production of two varieties of lentils at two stages of growth at Melfort.

Stage	S rate (kg S/ha) 0	10	20	40
		(µgS/g)		
Flower	2518	2792	2903	3265
Straw	3149	3337	3394	3995
Seed				
Eston	1916	1959	1991	2078
Ind'hd	1787	1885	1902	1950

TABLE 2 : Total sulphur content of lentils at Melfort at two stages of growth. Flower and straw values mean of Eston and Indianhead.

no response to S by either variety. Applications of N resulted in small increases in the total dry matter but seed yields were unaffected. The mean yield of Eston was 880 kg seed/ha and the mean yield of Indianhead was 551 kg seed/ha.

In the plant at flowering and in the straw at harvest the concentration of S was the same in both varieties (Table 2). Total S was lower in the seed produced by Indianhead than in Eston's seed. Applications of S fertilizer increased the total S in both varieties at all stages. Concentrations of SO_4-S in the plant tissue followed the total S concentrations and accounted for most of the increase in total S observed. Uptake of S by the plant increased between flowering and harvest. The continued increase in SO_4-S concentration indicated that the soil's S supplying power was in excess of the amount needed to meet plant growth requirements.

Applications of N had no effect upon nutrient content but it was observed that like the S uptake, N uptake increased between flowering and maturity.

At Loon Lake, just as in Melfort, Indianhead lagged behind Eston in dry matter accumulation during the vegetative stage but by harvest at maturity, Indianhead yields were only slightly less than Eston (Fig 2). Again, as at Melfort, the Indianhead would have continued to increase in total dry matter if the growing season had been longer.

Despite the low levels of available S in the soil at Loon Lake, applications of S had no effect on the total dry matter at any stage nor upon the seed yield of either variety.

Applications of N significantly increased dry matter yields of both varieties. Seed yield of Eston was also increased by N applications (Fig 3). Seed yield of Indianhead was so low that reliable estimates of treatment effects on yield were not possible. Responses to N were almost linear to the highest rate of N indicating a potential for higher yields existed with a better N supply. Like many pulse crops, to obtain highest yields, lentils

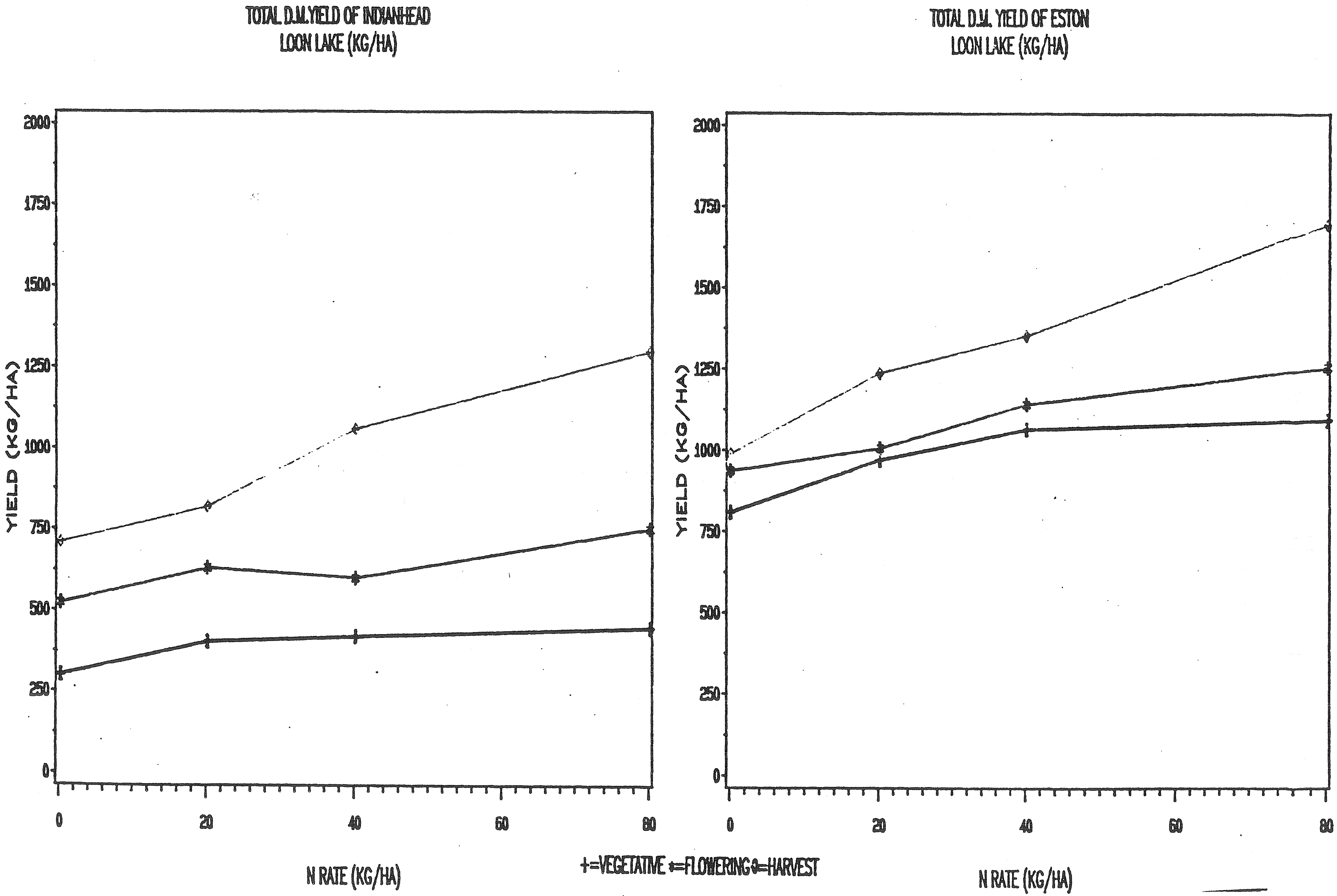

Fig 2: Effect of N applications on dry matter production of two lentil varieties at three stages of growth at Loon Lake.

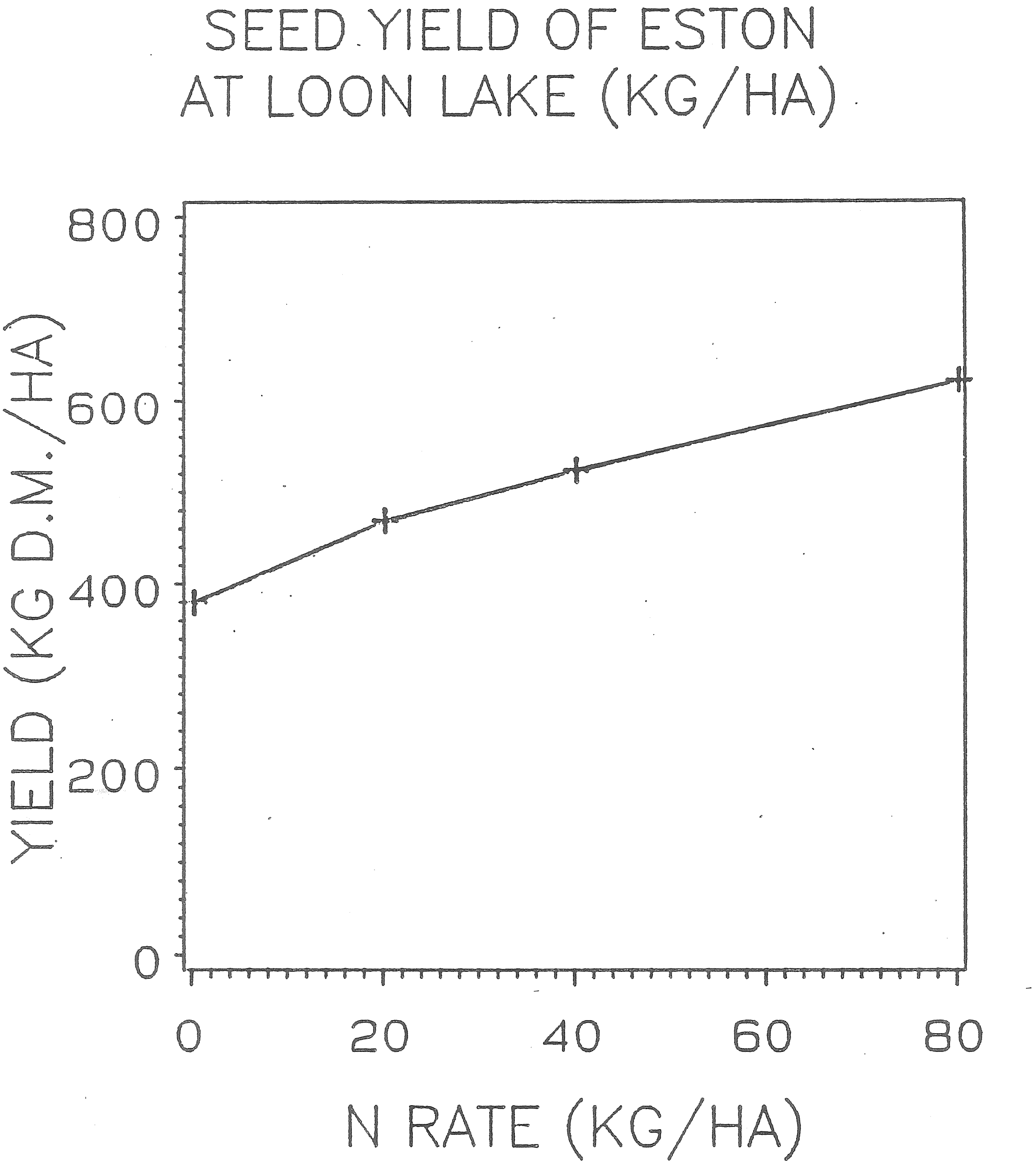

Fig 4: Effect of N application on the seed production of Eston lentils at Loon Lake.

Rate of S (kg S/ha)	Rate of N (kg N/ha)			
	0	20	40	80
	(μg S/g)			
0	1764	1440	1510	1354
10	2319	1946	1689	1500
20	2645	2409	1824	1807
40	3720	3440	2425	2301

TABLE 3 : Effect of N application and S application on the total S concentration in lentil straw at Loon Lake. (mean of two varieties)

require N to supplement the N they fix in their nodules. This may be the reason for the response to N observed here. Nodulation was not checked for extent or effectiveness.

There was a significant NxS interaction affecting the total S and the SO_4-S concentrations. At all stages and in both varieties S applications increased total S and SO_4-S and N applications decreased them. The response was similar at all growth stages and for total S and SO_4-S so only total S in the straw at harvest is shown (Table 3).

The soil at Loon Lake was very low in available S and in total S and had produced S deficiency in a prior rapeseed crop. Nevertheless, no adverse signs of S stress were noted in the lentil plants even when aggravated by N applications. This suggests that although the total amount of S available to the plant was limited, its small size ensured that the S concentration in its tissues would be high enough to prevent S deficiency occurring. It must be recognized, however, that other factors may have limited the size of the plant and that should these factors be removed, a bigger plant would result that may suffer from S deficiency.

Pot study

The poor S supplying power of the soil used in this study was confirmed by the response of the rapeseed plants. Responses similar to those described by Janzen and Bettany (1984) were observed. Where S was not applied, the plants were stunted, had the purpling characteristics of S deficiency, and flowered poorly. A few empty pods were produced in the 0S,300N treatment but there was no seed yield. At the 45S,300N treatment an average of 100 pods/pot were produced yielding 2.615g seed/pot. One pot, treated with 15S and 0N was seeded to rapeseed by mistake. This pot produced 22 pods and 0.651g seed.

In contrast to the rapeseed response, the lentils were not affected by the lack of S in the soil and did not respond to S applications in dry matter yield, seed yield, or root weight. There

Stage		N rate (µgN/g soil) 0	33	100	300
			(g/pot)		
Flowering		2.57	3.18	4.89	5.33
Mature(total)		3.64	4.36	6.01	8.22
Seed	0S	1.25	1.33	1.53	0.95
	5S	1.20	1.57	1.67	0
	15S	1.37	1.64	1.42	0
	45S	1.40	1.70	1.38	0

TABLE 4 : Effect of nitrogen applications on the dry matter production and seed yield of lentils in a pot study.

were no interactions between N and S for these factors but the plants did, however respond to N both in dry weight and seed yield (Table 4). At the 300N rate maturity was delayed and these plants were allowed to grow for several weeks after the other treatments had reached maturity. The 0S,300N treatments appeared to mature and yields were obtained that were lower than the yields from the other treatments. No seed was set at the other S rates as these plants were still in the flowering stage when they were harvested.

In both cuts, there was a significant NxS interaction affecting both the total S (Fig 4) and the inorganic plant S as measured by the HI-S method (Fig 5) and the extractable SO4-S method. There was however no difference in S levels betwen the cuts. As was seen in the Loon Lake test, increasing the S level in the soil increased the S level in the plant. Increasing the N level decreased the S level in the plant. At the 0S,300N treatment, total S was 700ppm and the HI-S level was 64ppm. At the 45S,0N treatment the mean total S was 5850 ppm and the HI-S was 4725ppm. Individual values of over 10000ppm were measured.

Fertilizer treatments had little effect upon the N content of the plant except at the 300N rate where high levels of NO_3-N were determined. This high level of NO_3-N was probably a result of luxury consumption. Total S and HI-S were almost as low at the 0S,100N rate but no NO_3-N accumulation was found. Nevertheless, this high a level may have interfered with protein synthesis causing the delayed maturity and the lower yield.

In this study the lentils have again been shown to be less susceptable than rapeseed to yield losses in low S soils. Selection over the centuries for plants that would survive and produce on poor soils have lead to a small plant that can therefore maintain a higher S concentration in the tissues.

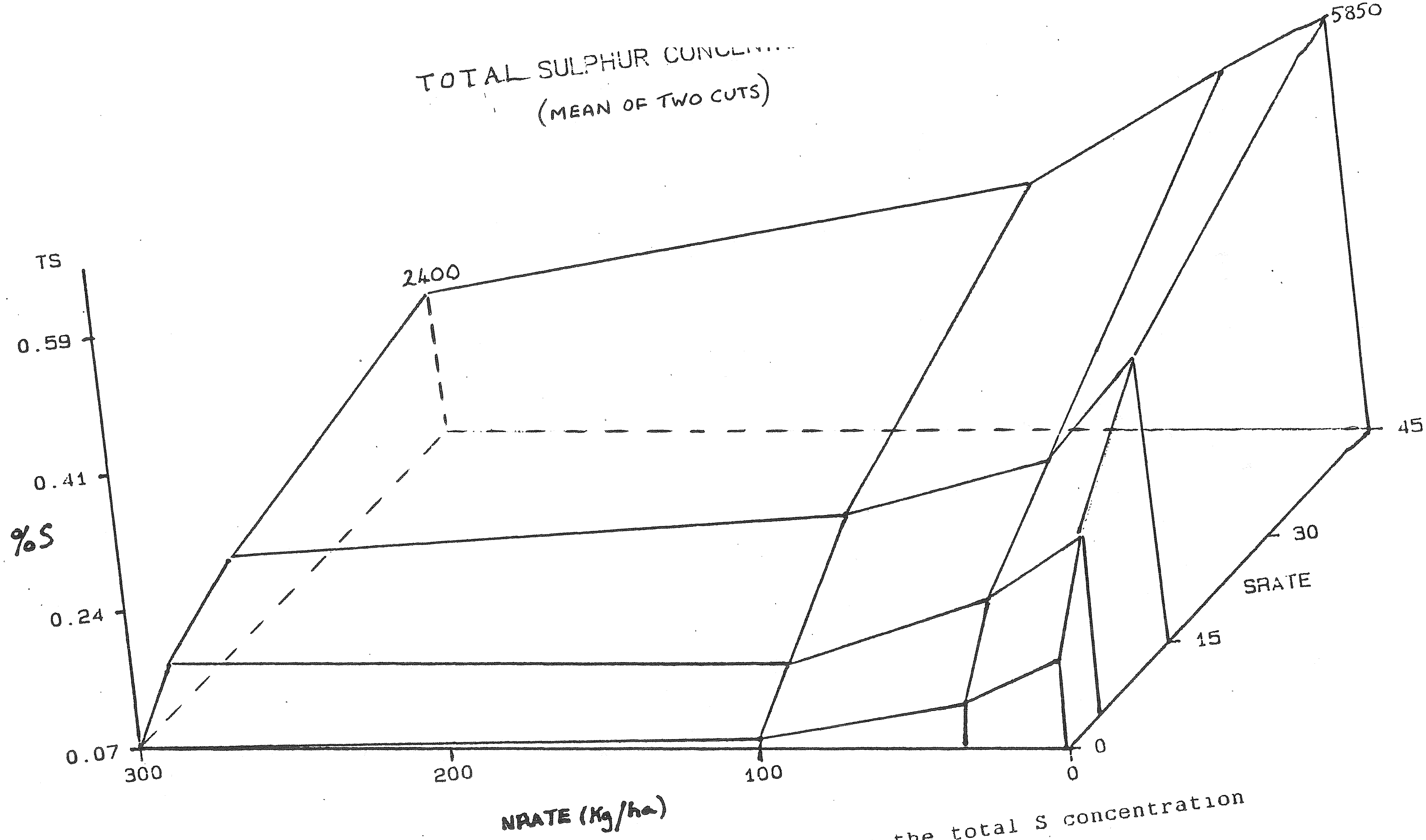

Fig 4: Effect of N and S applications on the total S concentration of Indianhead lentils in the growth room.

Diagnostic Criteria

Neither of the field trials showed S deficiencies and only one treatment in the pot study showed possible S deficiency so determination of diagnostic criteria is difficult. Nevertheless, these data can be used to indicate limits beyond which S deficiency is likely. Only criteria based upon seed analysis will be discussed.

N:S ratios in the seed from Melfort ranged from 14.8 to 17.7 and from 14.1 to 19.0 for Indianhead. These may be regarded as the normal range. At Loon Lake the ratios for Eston (too little Indianhead seed obtained for analysis) ranged from 14.6 to 21.9 reflecting the greater potential for S deficiency on this soil. In the pot study the N:S ratio's, with one exception, were between 13.9 and 17.3. All these ratios fall in the range of N:S ratios reported by Janzen and Bettany(1984) and are close to the 15:1 ratio present in protein (Dijkshorn and van Wijk, 1967; Stewart and Porter, 1969).

The one exception in the pot study occurred with the 0S,300N treatment. The N:S ratio of the seed was 42.6:1. Total N for this treatment was very high (5.05%) and reflects the interference of NO_3-N in the Kjeldhal determination. Nevertheless, even using a value of 3.5% (mean of other %N's) still results in an N:S ratio of 29:1. This indicates that these seeds were probably produced by a S deficient plant and accounts for the lower yield.

The alternative diagnostic is the determination of the decline of plant sulphate-S below a species specific limit. To bring this to a common denominator and in line with the prognostic indices produced by Maynard et al (1983), a ratio is determined.

The SO_4-S:total S ratio for lentil seed in Melfort varied from 0.46 to 0.77. At Loon Lake they varied from 0.35 to 0.72. In the pot study the ratios ranged from 0.31 to 0.46 with one exception. At the 0S,300N treatment the ratio was 0.09.

Thus we may conclude that lentil seeds with an N:S ratio higher than 22 and a SO_4-S:total S ratio below 0.31 were probably produced by a plant that was S deficient.

In the pot study, the inorganic S or ester bonded S was measured by extraction as SO_4-S and by hydriodic acid reduction (HI-S). HI-S determinations are considered more accurate and sensative than the extraction method. Comparison of the results of the two methods using over 200 data points showed that there is a linear relationship:

$$HIS = -470.5 + 1.028\ SO_4\text{-}S$$

Other workers have used the HI-S value to determine prognostic and diagnostic criteria. However, its determination is laborious and time-consuming. Since the two measurements are closely related, the ease of use of the SO_4-S method for extension purposes more than compensates for its poorer accuracy.

CONCLUSIONS

1. On soils with low levels of available N nitrogen fertilizer

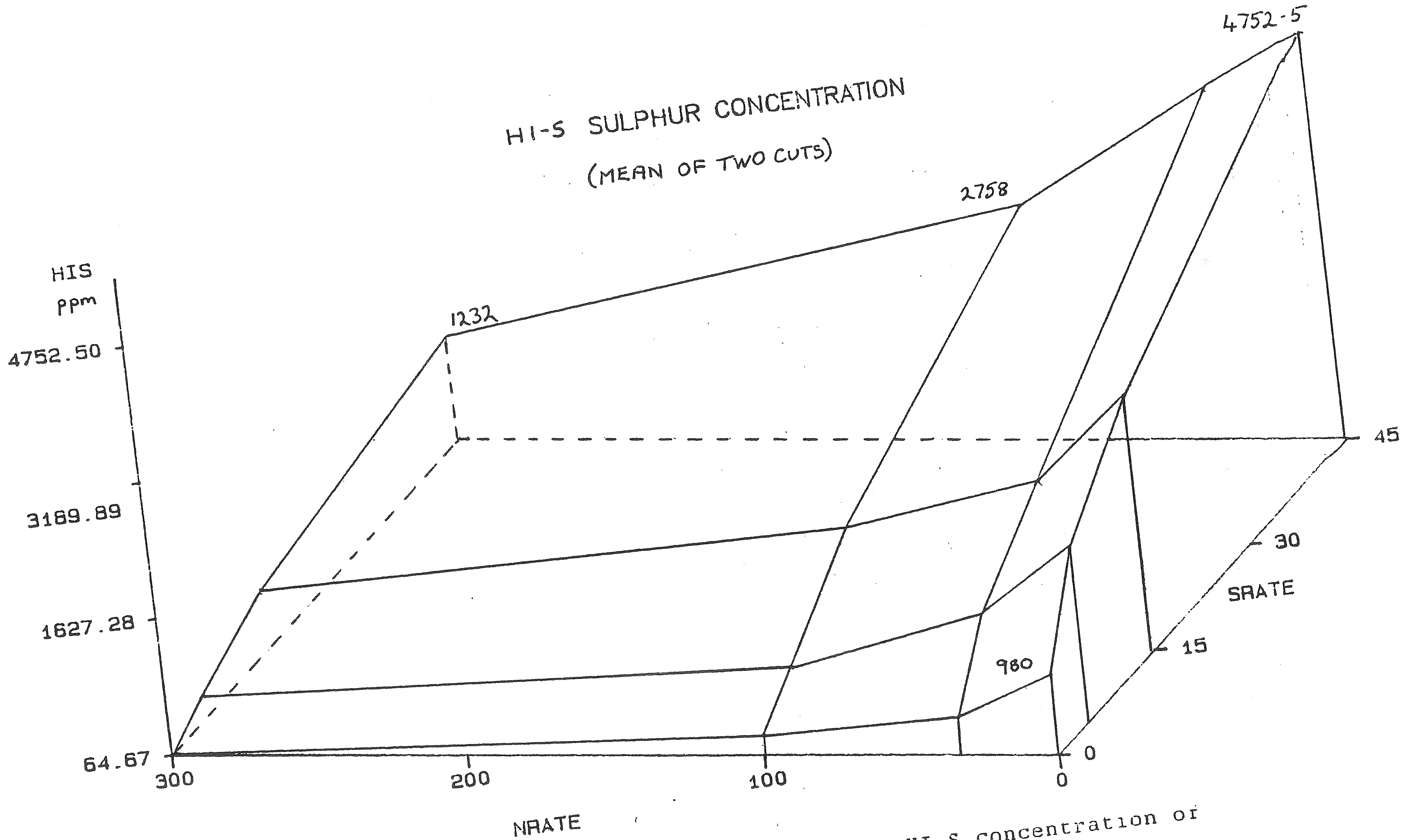

Fig 5: Effect of N and S applications on the HI-S concentration of Indianhead lentils grown in the growth room.

should be used to maximize yields.

2. Indianhead lentils should be seeded as early as possible to ensure a long growing season for maximum dry matter production.
3. Lentils will grow on soils with low levels of available S but excess N fertilizer may cause a S deficiency.
4. Diagnostic criteria based on seed analysis indicate S deficiency when the N:S ratio is above 22 and the SO4-S:total S ratio is below 0.3
5. Extractable plant SO_4-S is linearly related to HI-S and could be used in tissue testing for S deficiency.

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