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Title: EFFECTS OF STAGE OF MATURITY ON PHOSPHORUS AND SULFUR CRITICAL LEVELS IN TRIFOLIUM SUBTERRANEUM L.

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Abstract approved:

Dr. Thomas L. Jackson

Fertilization with phosphorus (P) and sulfur (S) is often necessary for production of subterranean clover (Trifolium subterraneum L.) in western Oregon. This study evaluated the use of plant analyses to predict response of subterranean clover to addition of these nutrients.

Field experiments were established in 1974 and 1976 to evaluate the effects of P and S on yield and chemical composition of subterranean clover. Source and rate of S and lime-P interactions were evaluated with clover grown on Nonpareil-Oakland and Veneta soils.

Total yield and P, S, and N concentrations were measured on mixed grass and clover forage harvested May 25 in 1976 and May 19 in 1977. Clover samples, consisting of leaflets and petioles, were collected at three week intervals from March 10 to May 25 in 1976 and two week intervals from March 18 to May 12 in 1977. Clover samples were analyzed for P, S, N, Mg, K, Ca, Mn, Zn, and Cu.

Yield increases from application of P ranged from 22 to 67%. Phosphorus concentrations of mixed forage increased with P fertilization from 0.14% to 0.20% P. Phosphorus concentrations of P-fertilized clover exceeded that of clover that had not received added P at every sampling date both years. Phosphorus concentrations in clover not receiving P fertilization were 0.22% from mid-March to mid-April and 0.18% in mid-May. Corresponding values for P-fertilized clover were

0.28% from mid-March to mid-April and 0.25% in mid-May. Phosphorus concentrations of clover from all treatments remained relatively stable from mid-March to mid-April and were not affected by lime or S treatments. The P critical level for clover collected between mid-March and mid-April was 0.25% P.

Sulfur fertilization increased yield 45% and more than doubled S concentration of mixed forage. Added S increased S concentrations of clover at every sampling date. Sulfur concentrations in clover that had not received added S were approximately 0.15% in late March and 0.08% in mid-May. Corresponding values for S-fertilized plants were 0.22% S and 0.20% S. Sulfur concentrations were relatively constant from mid-March to mid-April, and 0.20% S was identified as the critical level for clover samples collected during this time.

The total N to total S, $(N:S)_t$, ratios ranged from 21 to 35 in S deficient clover and from 16 to 27 in S-fertilized clover. The apparent critical $(N:S)_t$ ratio was 28.

Protein and nonprotein components of clover were estimated using ethanol and trichloroacetic acid (TCA). The N:S ratio in the insoluble (protein) fraction was approximately 30 to 35 from March 18 to May 12 in 1977. Ethanol-soluble and TCA-soluble (nonprotein) N concentrations remained fairly constant at about 80% of the total N regardless of level of S fertilization or stage of maturity. Ammonium-N and nitrate-N comprised about 0.07 and 0.04% of the plant, respectively.

Ethanol and TCA extracted different amounts of S from clover. Ethanol extracted an average of 43% of the total S from S-fertilized plants, while TCA extracted an average of 60% from the same samples. Both extractants removed an average of 36% of the total S from S deficient clover. If these results were valid, they indicate presence of nonprotein S in immature subterranean clover regardless of S status or stage of maturity.

These data suggest further research to investigate 1) relationships between protein synthesis and accumulation of nonprotein N and S in subterranean clover and 2) chemical procedures necessary to evaluate these processes.

Effects of stage of maturity on phosphorus and sulfur critical levels in *Trifolium subterraneum* L.

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INTRODUCTION

Trifolium subterraneum L., commonly called subterranean clover or subclover, is an important legume in areas of the world characterized by warm, moist winters and dry summers. It is especially well-adapted to the hill pastures in western Oregon, where it is used extensively for grazing sheep and cattle. In this area subterranean clover has allowed previously marginal land to support a prosperous livestock industry.

Subterranean clover is productive on steep, shallow, moderately acid soils, but the addition of phosphorus and sulfur is needed for establishment and optimum growth on most of the non-cropland pasture soils in western Oregon. The expense of applying these fertilizers makes it important to develop better methods to determine the P and S status of this plant to provide a basis to predict response from these nutrients.

Research has shown that plant tissue analysis can be used to assess the nutrient status of plants. The nutrient content of plant tissue can be correlated with yield increases from fertilization over a wide range of soil types (Melsted and Peck 1973; Tisdale 1966).

The chemical composition of plants varies greatly with plant part sampled and stage of maturity. Tissue selected and time of sampling must be specified when identifying a critical level, that nutrient concentration adequate for optimum yield (Ulrich 1952). Phosphorus and S critical levels have been proposed for subterranean clover samples collected at flowering (Jackson 1972; Jones 1974), but the relationships between plant maturity and nutrient content have not been investigated under Oregon conditions.

The diagnostic value of plant analysis for subterranean clover may be improved by establishing critical levels for earlier stages of

growth and using N:S ratios to provide a measure of S deficiency.

The objectives of this study were as follows:

1. to evaluate effects of P and S fertilization on yield of subterranean clover,
2. to evaluate effects of P and S fertilization on nutrient concentration of subterranean clover at different stages of growth,
3. to select a range of times to sample clover plants that would minimize changes in P and S concentrations due to maturity, and
4. to determine if N:S ratios could be used to identify need for S fertilization.

REVIEW OF LITERATURE

Subterranean Clover

Subterranean clover is a productive forage legume adapted to mild winter temperature and dry summers. As a winter annual this clover germinates after fall rains and makes maximum growth during the spring. Seed set occurs before the summer drought and is followed by plant senescence.

Subterranean clover can be established where topography prevents seedbed preparation (Morely 1961; Rampton 1952). Soil acidity as low as pH 4.5 can be tolerated (Pearson 1964; Williams 1957), and lack of nitrogen in the soil can be overcome by symbiotic N fixation (Morely 1961; Walker et al. 1956a; Walker et al. 1956b).

During a spring growing season, well-managed subterranean clover and grass fields can yield over three oven-dry tons of high quality, palatable livestock feed per acre (Jackson 1972; Jackson et al. 1964; Morely 1961). Subterranean clover persists since it has the unusual ability to bury seed in the ground, thus insuring future stands under heavy grazing pressure (Francis et al. 1976; Jones et al. 1972; Southwood 1976; Williams et al. 1957).

Subterranean clover and grass are generally grown together in pastures. The presence of the clover stimulates grass production through increased N fertility (Ozanne et al. 1976) and thus increases nutritional value of the grass (Jones 1976). Seasonal distribution of forage is improved by providing earlier fall growth and more winter production of grasses from the higher level of N present (Baylor 1974; Jones 1974; Jones 1976; Willoughby 1953). In addition, perennial grasses associated with this production system produce more early summer feed than annual grasses present on unimproved ranges.

Phosphorus

Responses by subterranean clover to P fertilization have been known for many years (Cooper 1958; Jackson et al. 1964; Rampton 1952). Phosphorus is necessary for chemical energy exchanges fundamental for growth and is found in numerous chemical forms in the plant (Tisdale and Nelson 1975). Fertilization of P deficient subterranean clover results in increased P content (Raguse and Evans 1977), increased nodulation (Osman et al. 1977), increased protein content (Jones 1974; Ozanne et al. 1976), and increased yield (Baylor 1974; Barrow 1975). Growth of associated grasses is improved, with corresponding increases in N and P uptake (Ozanne et al. 1976) and digestibility of the forage (Jones 1974). Phosphorus fertilization also affects the botanical composition of the pasture (Anderson and McLachlan 1951; Baylor 1974; Guerrero and Williams 1975; Ozanne et al. 1976; Rossiter 1964), generally favoring clover at moderate levels and grasses at high levels.

Critical levels based on plant analyses are identified by comparing a measure of nutrient uptake, such as the concentration of P in the plant, to yield responses to fertilization. The critical level is the percentage of P just sufficient for maximum yield (Ulrich 1952). Stage of maturity and selection of the plant part sampled must be specified, for the concentration of P is known to vary with these factors in many species (Martin and Matocha 1973). These variations can obscure the differences in P concentration due to nutrient availability and complicate establishment of meaningful critical levels.

The P concentration of subterranean clover decreases as the plant matures (Griffith 1974; Jones 1976; Jones et al. 1972; Raguse and Evans 1977). Experiments conducted under greenhouse conditions in California showed that varietial differences in P uptake can be significant in subterranean clover (Jones et al. 1970b) and that clover stems contained lower concentrations of P than petioles of leaves (Jones et al. 1972). It was concluded from greenhouse experiments

in California and field experiments in Australia that defoliation prior to sampling can increase P critical levels for subterranean clover (Jones et al. 1972; Ozanne 1971).

Jones (1974) concluded that P plant analysis had limited value for predicting the P status of subterranean clover plants. However, this conclusion was based on greenhouse studies where the plants do not go through a near dormant period as they do under field conditions. Differences in temperature and plant growth between greenhouse and field conditions may affect P critical levels.

Critical P levels of approximately 0.25% in leaves and petioles at harvest in June have been proposed for subterranean clover based on field studies in Oregon (Cooper 1958; Jackson 1972; Jackson et al. 1964). However, the effects of variety, stage of maturity, plant part, and presampling defoliation were not evaluated in these studies.

Sulfur

Response of legumes to S fertilization has been frequently observed in western Oregon (Jackson 1972; Rampton 1952). Adequate S, necessary for protein synthesis, is required to maintain yield and forage quality in subterranean clover (Anderson and Spencer 1950; Griffith 1974; Jackson 1972; Jones et al. 1970a). Fertilization of S deficient clover results in increased S, N, protein and yield in the associated grasses as well as the legume (Anderson and Spencer 1950; Bouma 1975; Jones 1963; Walker and Adams 1958; Walker et al. 1956a). Added S also increases the competitive ability of subterranean clover with respect to grasses, thus affecting the species composition of the pasture (Jones 1963; Jones et al. 1970b; Walker and Adams 1958).

Plant tissue analyses are widely accepted as indicators of S status (Bouma 1975; Tisdale 1966; Westermann 1975a). The value of plant analysis for diagnosis of S deficiency has been established by work on alfalfa where a total S concentration of 0.22% is considered the critical level for plant tops harvested at early bloom (Pumphrey

and Moore 1965a; Westermann 1975a).

Similar total S values have been reported for subterranean clover in Australia (Andrews 1975; Bouma et al. 1969), but critical S values for field conditions have not been established in the United States (Jones 1974).

In a grass-clover association, the yield of clover is frequently limited by S, while the yield of the grass is limited by N. Grass is more efficient in S uptake than clover, absorbing 96 to 98% of the S available from mineralization in the soil (Jones 1963; Walker and Adams 1958), and can contain sulfate levels indicative of luxury consumption while the clover is S deficient. The increase in yield and S concentration following S fertilization is much greater in the clover than in the grass; thus, analysis of the clover provides the best indication of the S status of the pasture. (Jones 1963; Metson and Collie 1972).

Time of sampling must be specified as the concentration of S generally decreases throughout the growth cycle of many plants. Pumphrey and Moore (1965a) noted a decline from 0.36 to 0.18% S as alfalfa progressed from six inches high to full bloom. Decline in total S appears independent of the level of fertility in both subterranean clover and alfalfa (Bouma et al. 1969; Pumphrey and Moore 1965a).

Nitrogen to Sulfur Ratios

Since N and S are both essential for protein synthesis, the ratio of these two elements has been investigated as an index of nutritional status. Several researchers have found the N:S ratio in the protein fraction of plants, $(N:S)_p$, to be relatively constant regardless of S nutritional level (Dijkshoorn et al. 1960; Stewart and Porter 1969; Stewart and Whitfield 1965). From a review of published data, Dijkshoorn and Van Wijk (1967) concluded that the (N:S) ratio was about 17.5 in legumes and 13.6 in grasses.

Sulfate S is taken up by the plant and converted to protein S. When protein synthesis is inhibited by a shortage of S, nonprotein forms of N accumulate and very little free sulfate remains in the plant (Dijkshoorn and Van Wijk 1967; Stewart and Porter 1969; Thompson et al. 1970). Where S is more than adequate to satisfy requirements for protein synthesis, luxury consumption of sulfate can occur (Dijkshoorn and Van Wijk 1967; Evans 1975; Thompson et al. 1970).

A total N to total S, $(N:S)_t$, ratio wider than the $(N:S)_p$ would indicate S deficiency or luxury consumption of N, while a narrower ratio would indicate N deficiency or luxury consumption of S. The $(N:S)_t$ ratios of S deficient plants are compared to $(N:S)_t$ ratios of plants receiving adequate S as the basis for critical levels.

The $(N:S)_t$ ratio has been used to identify S deficiency in a number of crops including alfalfa (Pumphrey and Moore 1965b; Reddy and Mehto 1970; Westermann 1975a), various grasses (Metson and Collie 1972), grass and clover pastures (Metson and Collie 1972; Walker et al. 1954) and corn, beans, and wheat (Stewart and Porter 1969). Pumphrey and Moore (1965b), in their work with alfalfa, observed that the percentage of N and S both declined throughout the growing season, but the $(N:S)_t$ for plants at a particular nutritional level remained nearly constant from the time the plants were six inches tall to full bloom. Thus, the $(N:S)_t$ ratio could be used as a diagnostic tool to identify S deficiency over an extended sampling period.

Little, if any, work has been done with $(N:S)_t$ ratios in subterranean clover. Total S and total N values were recorded by Anderson and Spencer (1950) for a greenhouse experiment in Australia. Although $(N:S)_t$ ratios were not calculated at the time, their relationships do indicate that the principles might hold for subterranean clover. In this experiment, the S deficient plants had an $(N:S)_t$ ratio of

$$1.71\% \text{ N} / 0.058\% \text{ S} = 29.3.$$

The S adequate plants contained

$$2.26\% \text{ N} / 0.244\% \text{ S} = 9.3.$$

As the expected $(N:S)_p$ for legumes is around 17.5, these results correspond to the expected relationship between the $(N:S)_t$ of S deficient and adequate plants.

While most studies empirically support the value of the $(N:S)_t$ ratio for diagnosing S deficiency (Metson and Collie 1972), not all support the basic premise that the $(N:S)_p$ ratio remains constant. Metson and Collie (1972) reported a range in the $(N:S)_p$ of white clover from 16.4 to 21.0, while Westermann (1975a) found ratios ranging from 17 to 23 in alfalfa. Environmental conditions, such as nutrient availability, can cause changes in the relative proportions of cytoplasmic proteins, though the amino acid sequence of particular proteins is not affected (Mertz and Matsumato 1956; Westermann 1975a). Jones et al. (1971) found an increase in the $(N:S)_p$ ratio of the tropical legume Townsville stylo with increasing S deficiency and increasing maturity.

MATERIALS AND METHODS

Response of subterranean clover to applications of P and S was evaluated with field experiments on two locations near Roseburg in Douglas County. The area near Roseburg is characterized by warm, moist winters and dry summers. The average annual temperature is 55°F and the frostfree growing season approximately 230 days. The mean annual precipitation is 32 inches, most of which falls in the winter.

The first experiment was established in September 1974, on the Foster farm about ten miles east of Roseburg and was located on a complex of Nonpareil and Oakland soil series. These soils are found on gentle to steep slopes in the interior valleys of the Umpqua Basin. The Nonpareil series, a member of the fine, loamy, mixed, mesic, shallow family of Dystric Xerochrepts, is characterized by a brown loam surface layer and a brown and dark yellowish brown loam subsoil. The Oakland series, a member of the fine, mixed, mesic family of Ultic Haploxeralfs, typically has a brown loam surface soil and a brown silty clay loam subsoil. Both of these soils developed from sedimentary parent material. Depth varies from 10 to 20 inches in the Nonpareil and 20 to 40 inches in the Oakland. The use of these soils for agricultural purposes is restricted by slope, depth to bedrock, and associated limitations on water supplying capacity and root penetration. Primary use is forage production.

The second experiment was established in September, 1976, on the Murphy farm about 13 miles northwest of Roseburg and was located on the Veneta soil series. The Veneta series, a member of the fine, mixed, mesic family of Ultic Haploxeralfs, is characterized by a dark brown silt loam surface layer and a brown clay to clay loam subsoil. This soil is well drained and has an effective rooting depth of 30 to 40 inches. Members of this series are used mainly for forage and small grain production.

Soil samples were taken from the surface six inches of soil in September prior to applying fertilizer treatments. Chemical

analyses (Table 1) were performed by the Oregon State University Soil Testing Laboratory according to published procedures (Kauffman and Gardner 1976; Roberts et al. 1971).

Both experimental sites were fenced to exclude sheep from October to May. Intensive grazing in June was used to remove regrowth and aftermath from all plots.

Table 1. Chemical analyses of soil samples from experimental sites.

Soil	Location	pH	SMP	CEC	Ca	Mg	K	P
				--- meq/100g---			--ppm-	
Nonpareil-Oakland	SW 1/4, Sec 28, T 26 S, R 4 W	5.8	6.3	14.1	7.9	2.2	250	16
Veneta	E 1/4, Sec 35, T 25 S, R 7 W	5.3	6.1	---	1.9	0.6	48	10

Field Experiment 1: Nonpareil-Oakland Soil

The pasture on the Nonpareil-Oakland soil complex had been planted to Nangeela subterranean clover some years earlier and had been grazed by sheep. Introduced annual grasses comprised most of the non-legume component. During the previous ten years 200 pounds of single super phosphate (8% P and 10% S) per acre had been applied every second year.

Soil analyses (Table 1) showed K, Ca, and Mg levels considered adequate for clover growth. Phosphorus levels were low and indicated a probable P response, while the soil pH and SMP buffer pH revealed moderate acidity within the range of subterranean clover tolerance.

A number of S rate and source treatments plus P check plots were established in a randomized block design with five replications. Results from eight of the treatments (Table 2) are discussed in this paper. All plots received a blanket application of two pounds of boron and one pound of molybdenum per acre to be sure that these nutrients were not limiting clover growth. Each plot was six feet wide and twenty feet long.

Table 2. Treatments applied to subterranean clover grown on the Nonpareil-Oakland soil complex.

Treatment	Rate P	Rate S	Source P	Source S	Schedule
	lbs/acre				
1. 26 P	26	--	MCP†	--	applied each fall
2. 26 P + 20 Sg	26	20	MCP	gypsum	applied each fall
3. 26 P + 40 Sg	26	40	MCP	gypsum	applied each fall
4. 26 P + 40 Sf	26	40	MCP	S°††	applied each fall
5. 26 P + 160 Sf	26	160	MCP	S°	P applied each fall S applied once in 1974
6. 26 P + 80 Sf	26	80	MCP	S°	P applied each fall S applied in 1974 & 1976
7. 0 P + 40 Sg	--	40	--	gypsum	applied each fall
8. 0 P + 40 Sf	--	40	--	S°	applied each fall

† monocalcium phosphate
†† elemental sulfur less than 0.25 mm in diameter

Field Experiment II: Veneta Soil

The Veneta soil location had been in subterranean clover and grass pasture previously and had received modest applications of single super phosphate to supply both P and S. Analysis of the soil (Table 1) indicated low levels of P and K and acidity within the tolerance range of subterranean clover but low enough to expect response from lime.

Treatment discussed in this presentation included a 3 x 3 lime x P factorial. Treatments were applied in a split plot design with lime applications as the main plots and P treatments as subplots. Each individual plot measured 25 by 7.5 feet.

The area was plowed, disced, and rolled, then treatments of 0, 2, and 4 tons of lime per acre were disced into the soil. Lime treatments raised the soil pH of samples taken the following March from 5.3 to 5.9 and 6.2 for 2 and 4 tons of lime per acre respectively.

Phosphorus treatments of 0, 17, and 51 pounds per acre were broadcast and the area replanted to Nangeela subterranean clover and Linn perennial ryegrass. All plots in this discussion received uniform application of boron, molybdenum, sulfur, and potassium at rates of 2, 1, 40, and 166 pounds per acre respectively.

Plant Sampling and Harvest

Plant samples from all plots of treatments 1, 2, 3, 5, and 7 were collected on the Nonpareil-Oakland site in 1976 at approximately three week intervals starting March 10; however, samples were collected from only two replications on treatments 1, 2, and 3 on April 22. Approximately 30 grams of fresh plant material were collected from each plot by random selection. The grass was separated from the clover and chemical analysis performed on a mixture of clover leaflets and petioles.

On the fifth sampling date, May 25, 1976, the forage was harvested. A self-propelled forage harvester was used to mow a 17 by 3.5 foot swath in the center of each plot. The fresh plant material from each swath was weighed and a moisture sample collected to calculate total dry matter yields. Samples of the grass and clover mixture, as well as clover leaflets and petioles, were saved for chemical analysis.

In 1977, plant samples were taken from both experiments at two week intervals starting March 18. Sampling was done in the same manner as in 1976, with five samplings being made before harvest on May 19. Harvest practices were identical, except that the swathes for Experiment II on the Veneta soil were 3.5 feet by 22 feet to accommodate the larger plot size. Clover leaflets and petioles were saved for analysis from the first five samplings, while a grass and clover mix was saved from the harvest to measure total uptake of P, S, and N.

Plant Analysis

Plant samples collected during each spring prior to harvest were dried for at least 48 hours at 60°C while the samples collected at harvest were dried at 70°C for at least 72 hours; a higher temperature setting was used on the larger moisture samples to reduce drying time. Clover samples consisted of a mixture of clover leaves and petioles, while samples of the mixed forage contained the whole tops of the clover and the grass. All dried samples were ground in a stainless steel Wiley Mill to pass a 20 mesh screen and stored in manila envelopes.

One gram of dried and ground plant material was digested with 12 mls of nitric acid and 6 mls of perchloric acid and analyzed for calcium (Ca), magnesium (Mg), potassium (K), manganese (Mn), and copper (Cu) using flame atomic absorption spectrophotometry. An aliquot of this digest was used to determine P by the vanadomolybdophosphoric acid colorimetric method (Jackson 1958).

Total N was determined by a Kjeldahl procedure (Jackson 1958). Total S was determined turbidimetrically as barium sulfate after dry ashing with magnesium nitrate (Appendix A) (Tabatabai and Bremner 1970; Westermann 1975b).

Analyses for protein N and S were performed on composite samples of treatments 1, 2 and 4 from subterranean clover grown on the Nonpareil-Oakland soil complex in 1977. These samples were ground to pass a 40 mesh screen, and two separate methods were used to extract the nonprotein fraction. A 0.3 gram sample of plant material was boiled with 33 mls of 70% (v/v) ethanol for 9 minutes, filtered, and washed with 66 additional mls of ethanol (Westermann 1975a). Nonprotein N and S were also extracted by shaking 0.3 gram of plant material with 10 mls of 5% (w/v) TCA (trichloroacetic acid) for 30 minutes, filtering, and washing with 10 mls of 5% TCA followed by 20 mls of distilled water (Bisset 1954). The residues from these extractions were assumed to be protein and were analyzed for N and S according to the total N and total S procedures above. Soluble

(nonprotein) N and S was calculated by difference.

Composite samples of undigested plant material were also analyzed for ammonium and nitrate N. A one gram sample was extracted by shaking for 30 minutes with 50 mls of 0.02% formic acid (Bremner and Keeney 1965). Ammonium and nitrate N were determined on the filtrate using a Kjeldahl procedure (Jackson 1958).

Statistical Analysis

An analysis of variance was performed on results from each sampling date. F tests and least significant differences (LSD) were computed at the 5% level.

RESULTS AND DISCUSSION

Chemical analyses of plant samples indicated there was adequate nutrition of all elements except P or S. Levels of K, Mn, Ca, Mg, Zn, and Cu in plant tissue from both experiments are recorded in Appendix B. Results from P and S analyses are discussed in separate sections below. Nitrogen analyses and (N:S) ratios are discussed in the third section.

Phosphorus

In both 1976 and 1977, dry matter yields of subterranean clover and grass grown on the Nonpareil-Oakland soil complex increased with P fertilization (Tables 3 and 4). In 1976, application of 26 P + 40 Sg (26 pounds of P per acre plus 40 pounds of S per acre as gypsum) resulted in a 22% yield increase over application of 40 Sg alone (Treatments 3 and 7, Table 3). Application of 26 P + 40 Sf (26 pounds of P per acre plus 40 pounds S per acre as finely ground elemental S) increased yield 65% over application of 40 Sf alone (Treatments 4 and 8, Table 3). In 1977, 26 P + 40 Sg increased yield 32% over 40 Sg alone, and 26 P + 40 Sf increased yield 67% over 40 Sf alone (Table 4).

The concentration of P in the mixed grass and clover forage grown on the Nonpareil-Oakland soil complex increased with added P. The P concentration in the forage that did not receive P fertilization was 0.14% both years, while P-fertilized plants contained 0.20% P in 1976 (Table 3) and 0.21% P in 1977 (Table 4). The differences in P concentrations in 1976 were not statistically significant, but were comparable to differences which were significant in 1977.

Phosphorus concentrations of the clover on this site increased with P fertilization throughout both growing seasons (Figures 1 and 2). Phosphorus concentration was relatively stable early in both seasons, from mid-March to mid-April, but decreased more rapidly near the end of April and in May. In 1976, plants that did not receive P

Table 3. Dry matter yield and P concentration of clover and grass forage grown on the Nonpareil-Oakland soil complex as affected by P and S fertilization. Harvested May 25, 1976.

Treatment			Total yield	nutrient concentration
	P	S†		P
	lbs/acre		tons/acre	%
7.	0	40g	2.91	0.14
8.	0	40f	2.19	----
3.	26	40g	3.54	0.20
4.	26	40f	3.62	----
		$LSD_{(.05)}$	0.49	n.s.

†g sulfur applied as gypsum
f sulfur applied as elemental S

Table 4. Dry matter yield and P concentration of clover and grass forage grown on the Nonpareil-Oakland soil complex as affected by P and S fertilization. Harvested May 19, 1977.

Treatment			Total yield	nutrient concentration
	P	S†		P
	lbs/acre		tons/acre	%
7.	0	40g	1.70	0.14
8.	0	40f	1.45	0.14
3.	26	40g	2.25	0.21
4.	26	40f	2.42	0.25
		$LSD_{(.05)}$	0.43	0.03

†g sulfur applied as gypsum
f sulfur applied as elemental S

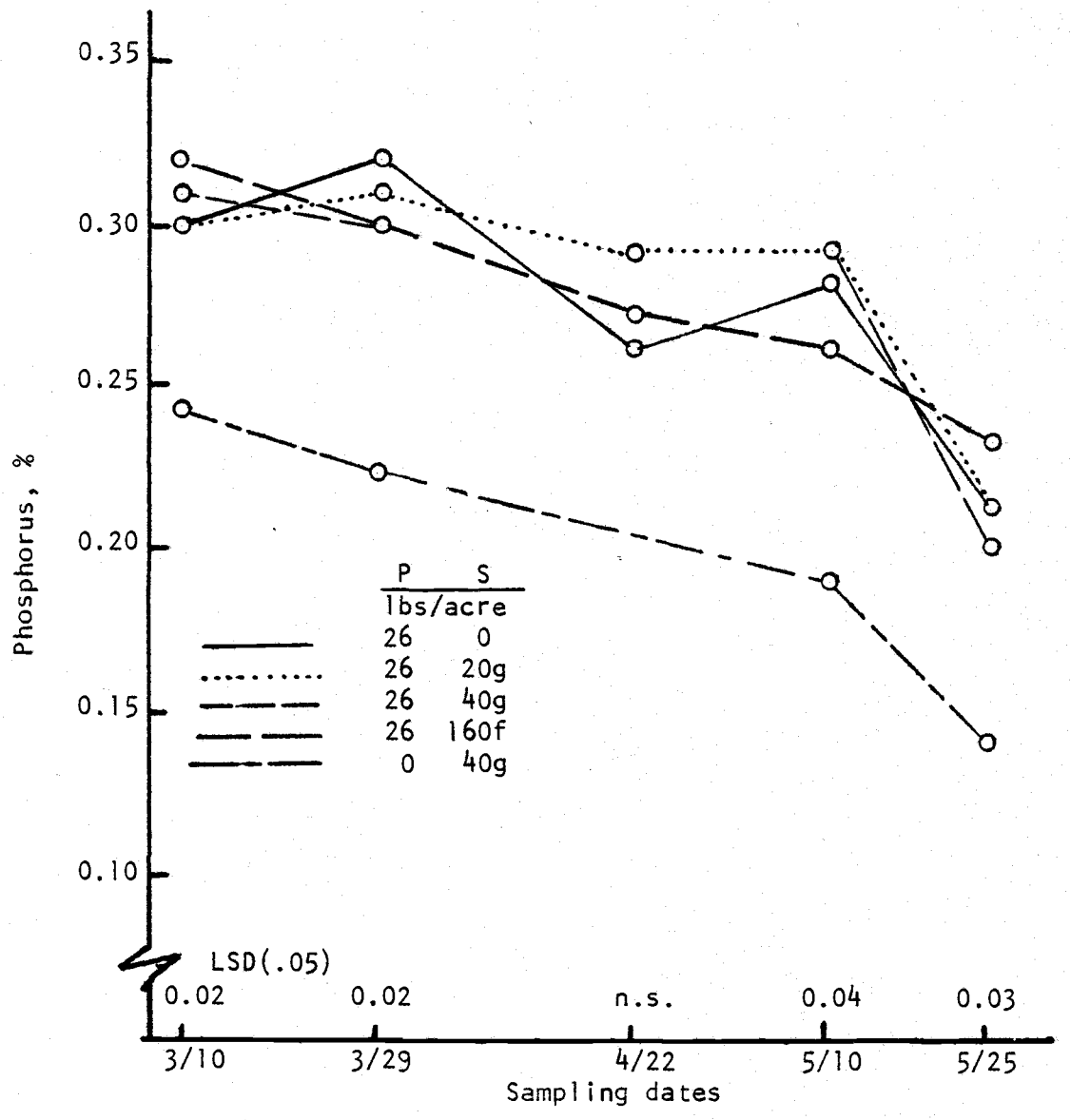

Figure 1. Phosphorus concentration of subterranean clover grown on the Nonpareil-Oakland soil complex as affected by P and S fertilization and plant maturity in 1976. (Sulfur applied as g-gypsum or f-elemental S)

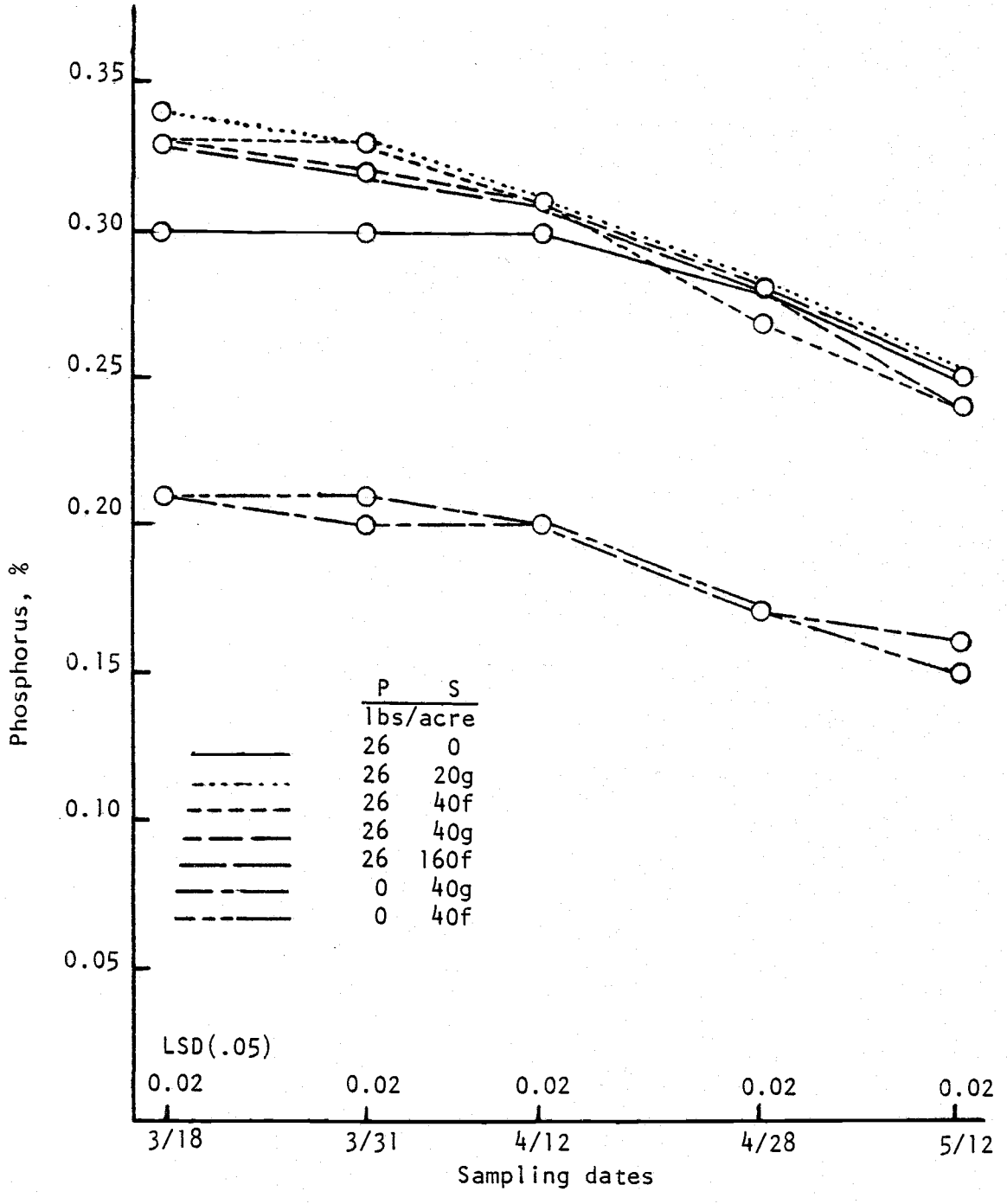

Figure 2. Phosphorus concentration of subterranean clover grown on the Nonpareil-Oakland soil complex as affected by P and S fertilization and plant maturity in 1977. (Sulfur applied as g-gypsum or f-elemental S)

fertilization contained 0.24% P on March 10 and 0.19% P on May 10 (Figure 1). Two weeks later, on May 25, the concentration had dropped to 0.14% P. Plants that had received P contained approximately 0.31% P on March 10, 0.28% on May 10 and 0.23% on May 25. In 1977, the P concentrations of all plants receiving all treatments were nearly constant from March 18 to April 12 (Figure 2). Plants from the P check plots contained 0.21% P during this period and 0.16% on May 12. Plants receiving P contained approximately 0.32% P from March 18 to April 12, and 0.25% on May 12. Phosphorus concentration was only slightly influenced in either year by application of S varying from zero to 160 pounds per acre.

Yield data for the grass and clover forage grown on the Veneta soil showed an increase of 28% with two tons per acre of lime plus 17 or 51 pounds of P (Table 5). Positive responses of clover to lime and P were evident earlier in the spring when clover made up the largest portion of the forage.

Application of lime increased yields, especially at low rates of added P, but did not affect the concentration of P found in the mixed grass and clover forage at harvest (Table 5). The concentration of P, an important consideration in livestock nutrition, was increased from 0.14 to 0.20% P in the mixed forage by fertilization with 51 pounds of P per acre.

Chemical analyses of clover samples collected every two weeks from the Veneta site showed that the level of P in the clover was not affected by lime treatments, but was affected by P fertilization (Figure 3). At all lime rates, clover not receiving P fertilization contained approximately 0.22% P on March 18 and 0.17% P on April 25. Phosphorus concentrations approximately 20 and 35% higher were found in clover receiving 17 P and 51 P, respectively, regardless of rate of lime application. As observed with clover grown on the Nonpareil-Oakland soil complex, there was a definite plateau in P concentration from mid-March to mid-April. These results suggested that analysis of samples collected at this time, when P values were relatively stable, could be used to identify critical levels for subterranean

Table 5. Dry matter yield and P concentration of clover and grass for age grown on Veneta silt loam as affected by P and lime rates. Harvested May 19, 1977.

Lime, tons/acre	P, lbs/acre 0	17	51
	Yield, tons/acre		
0	2.28	2.64	2.82
2	2.85	2.93	2.93
4	2.83	2.57	2.57
	P, %		
0	0.14	0.17	0.21
2	0.14	0.18	0.23
4	0.15	0.19	0.20

$LSD_{(.05)}$ for yield = 0.58 tons/acre
for P = 0.04 %

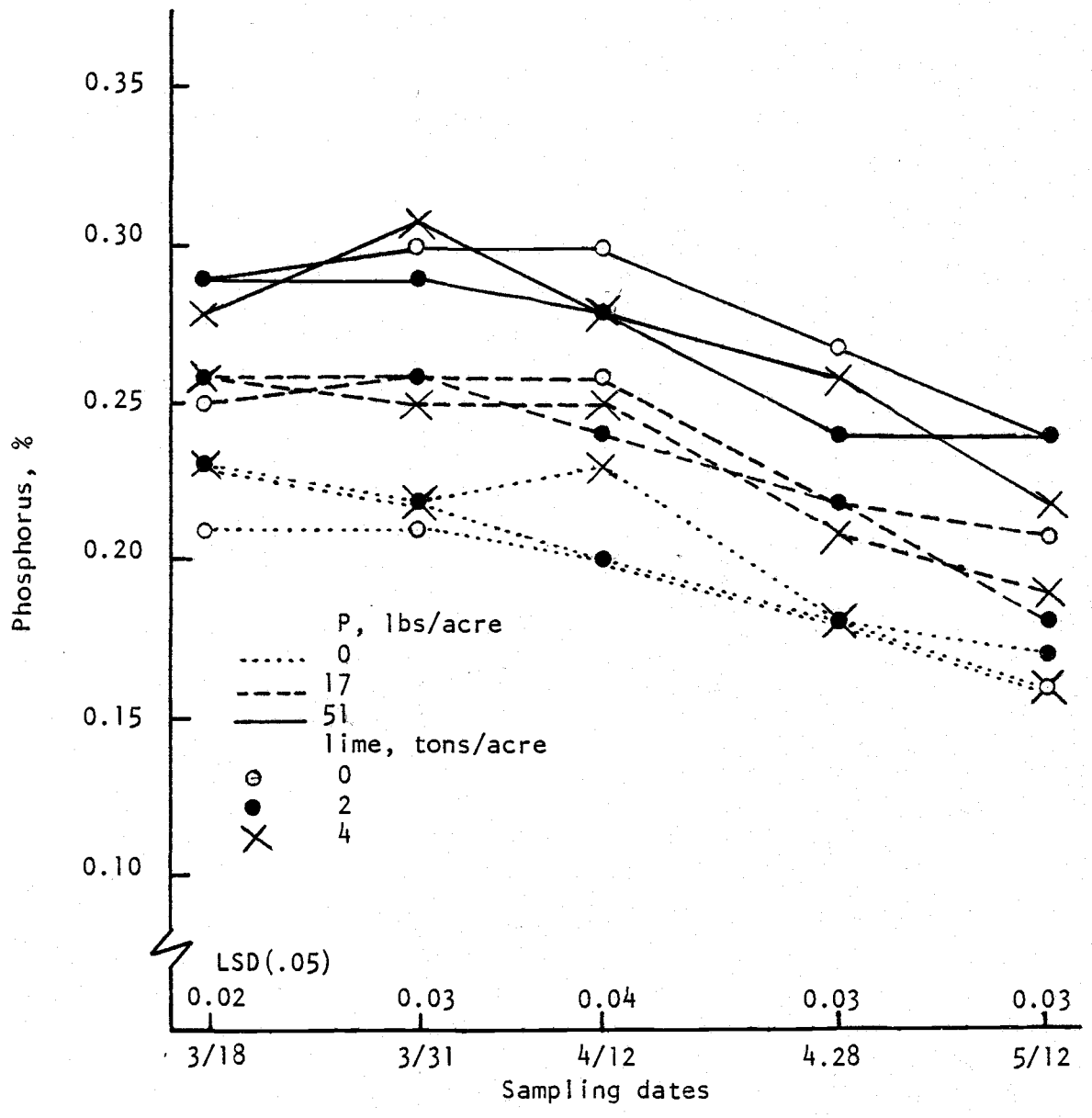

Figure 3. Phosphorus concentration of subterranean clover grown on Veneta silt loam as affected by lime, P treatments, and plant maturity in 1977.

clover.

A concentration of 0.25% P in clover collected from the Veneta site between mid-March and mid-April corresponded to maximum yield at harvest. Since plant analysis values for the clover receiving 26 P on the Nonpareil-Oakland soil complex were higher than those of plants receiving 51 P on the Veneta soil, it was assumed that the 26 P treatment provided adequate P in that experiment for maximum yield. Results from the experiment on the Nonpareil-Oakland soil complex also indicated that a concentration of 0.25% P in clover samples collected between mid-March and mid-April was the minimum level found in plants producing maximum yield and could be used as the critical level for P in subterranean clover.

Sulfur

Sulfur relationships in subterranean clover were examined on plants grown on the Nonpareil-Oakland soil complex in 1976 and 1977. Yield data from both years showed that all treatments that supplied 26 P plus 40 or more pounds of S per acre increased yield 45% over 26 P without S (Tables 6 and 7). Application of 26 P + 20 Sg or 40 pounds of S without P did not supply adequate nutrition for maximum yield either year.

Sulfur fertilization increased the concentration of S and N in the mixed clover and grass forage at harvest (Tables 6 and 7). The most effective S treatment (26 P + 40 Sf) more than doubled the S concentration both years, from 0.06 to 0.16% S in 1976 and from 0.08 to 0.21% S in 1977. Application of 40 S without P resulted in S concentrations significantly less than maximum in 1976 and equivalent to maximum in 1977. The nitrogen concentration appeared to increase with S fertilization, although the difference between 26 P alone and 26 P + 40 Sf or 26 P + 40 Sg was not statistically significant. In 1977, the highest N concentration was found in the mixed grass and clover samples from the 26 P + 20 Sg treatment. As the N concentration of the clover receiving this treatment was no higher at other sampling

Table 6. Dry matter yield and S and N concentration of clover and grass forage grown on the Nonpareil-Oakland soil complex as affected by P and S fertilization. Harvested May 25, 1976.

	Treatment		Total	nutrient concentration	
	P	S†	yield	S	N
	lbs/acre		tons/acre	%	%
1.	26	0	2.43	0.06	1.68
2.	26	20g	3.02	0.09	1.77
3.	26	40g	3.54	0.12	1.97
4.	26	40f	3.62	0.16	----
5.	26	160f	3.37	0.13	1.78
6.	26	80f	3.22	0.12	----
7.	0	40g	2.91	0.08	1.65
8.	0	40f	2.19	0.11	----
		$LSD_{(.05)}$	0.49	0.03	n.s.

†g sulfur applied as gypsum
f sulfur applied as elemental S

Table 7. Dry matter yield and S and N concentration of clover and grass forage grown on the Nonpareil-Oakland soil complex as affected by P and S fertilization. Harvested May 19, 1977.

	Treatment		Total	nutrient concentration	
	P	S†	yield	S	N
	lbs/acre		tons/acre	%	%
1.	26	0	1.64	0.08	2.07
2.	26	20g	1.95	0.16	2.68
3.	26	40g	2.25	0.18	2.34
4.	26	40f	2.42	0.21	2.48
5.	26	160f	2.41	0.20	2.37
6.	26	80f	2.39	----	----
7.	0	40g	1.70	0.19	1.91
8.	0	40f	1.45	0.21	2.07
		$LSD_{(.05)}$	0.43	0.04	0.47

†g sulfur applied as gypsum
f sulfur applied as elemental sulfur

dates than that of 26 P + 40 Sg, it was suggested that the proportion of species was affected by this treatment. Limitations on N fixation due to lack of S could decrease N available for grass growth and thus decrease the proportion of grass in the plot, resulting in higher total N.

Clover that received P but no added S contained lower concentrations of S at every sampling date than plants that received adequate P and S fertilization (Figures 4 and 5). In 1976, plants that did not receive added S contained 0.21% S on March 10 and 0.07% S on May 25. Sulfur concentrations in plants that received 26 P plus 40 or 160 S were approximately 0.25% S on March 10 and 0.20% S on May 25. In 1977, plants that did not receive added S contained 0.14% S on March 18 and 0.08% S on May 12. Plants that received added S contained 0.17 to 0.27% S throughout the season.

The increase in S concentration on April 22 could not be explained. Presumably, favorable conditions for soil microbiological activity must have resulted in a release of S and increased plant uptake at that time. However, sampling was not complete on that date.

Previous studies (Bouma et al. 1969; Jones 1964) showed that S concentration in subterranean clover declined with maturity and that sampling time was important in identifying critical levels. As the S concentrations in the study under consideration appeared most stable early in the season, S critical levels were established for samples collected between mid-March and mid-April. Plants containing over 0.20% S during this period produced maximum yield at harvest. In the future, plants containing S concentrations lower than this value could be expected to respond to S fertilization.

Nitrogen to Sulfur Ratios

In 1976, the N concentrations in clover receiving 26 P but no S were 4.8 to 4.4% from March 10 to May 10, then decreased to 2.4% on May 25 (Figure 6). The N concentrations in clover that received 26 P + 40 Sg were approximately 5.2% from March 10 to May 10 and decreased to 3.8% on May 25. Intermediate concentrations were found in plants

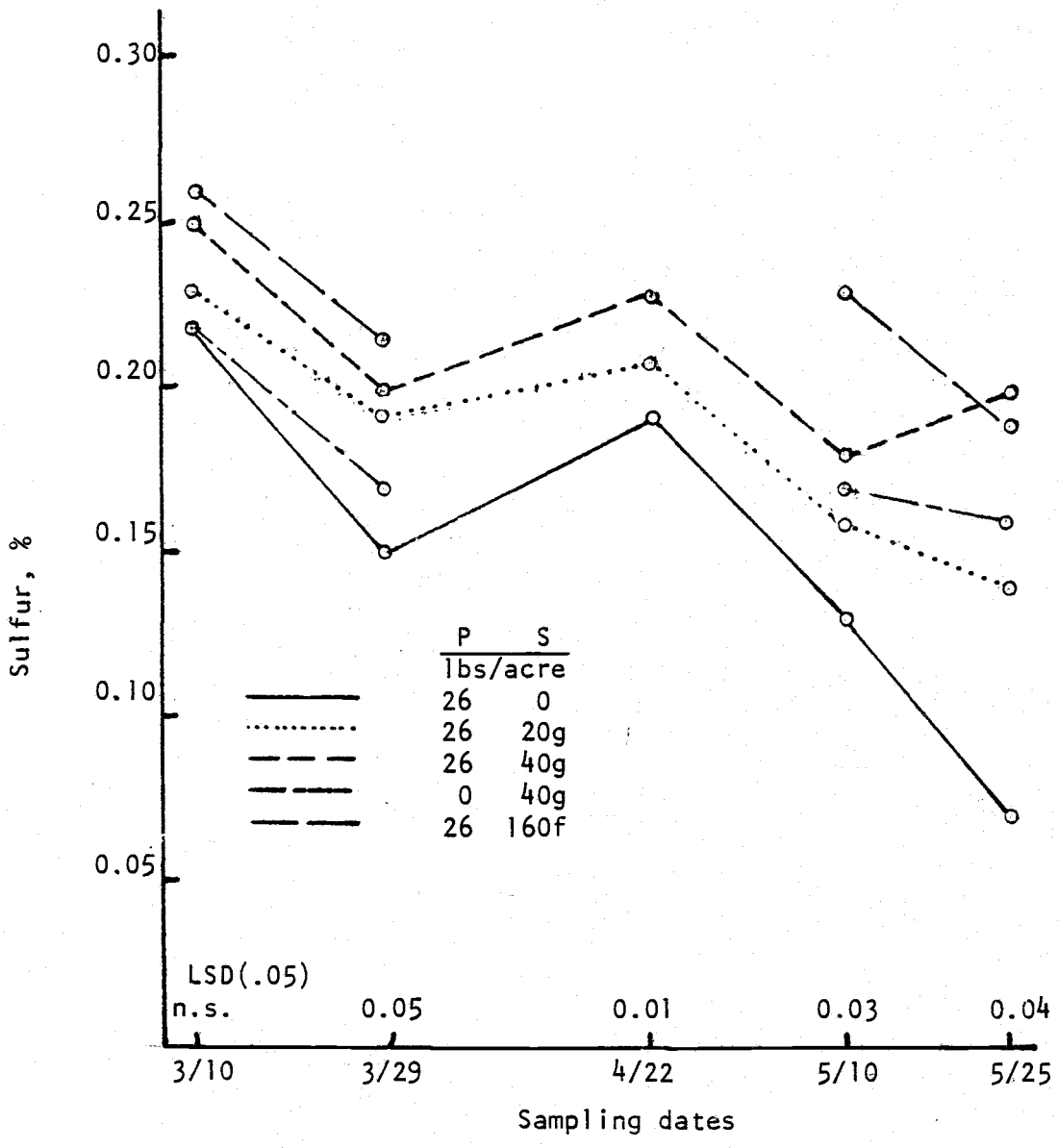

Figure 4. Sulfur concentration of subterranean clover grown on the Nonpareil-Oakland soil complex as affected by P and S fertilization and plant maturity in 1976. (Sulfur applied as g-gypsum or f-elemental S)

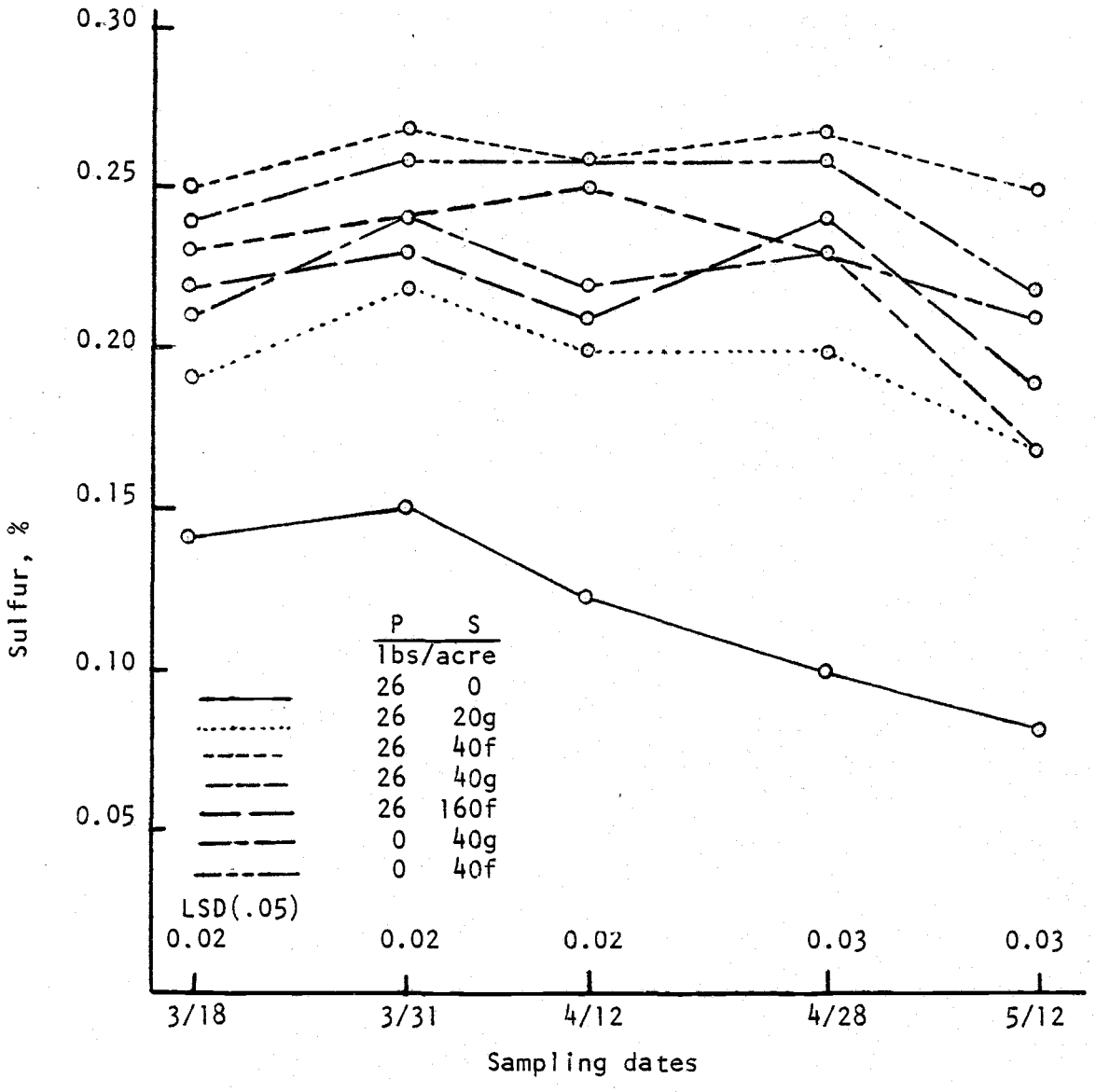

Figure 5. Sulfur concentration of subterranean clover grown on the Nonpareil-Oakland soil complex as affected by P and S fertilization and plant maturity in 1977. (Sulfur applied as g-gypsum or f-elemental S)

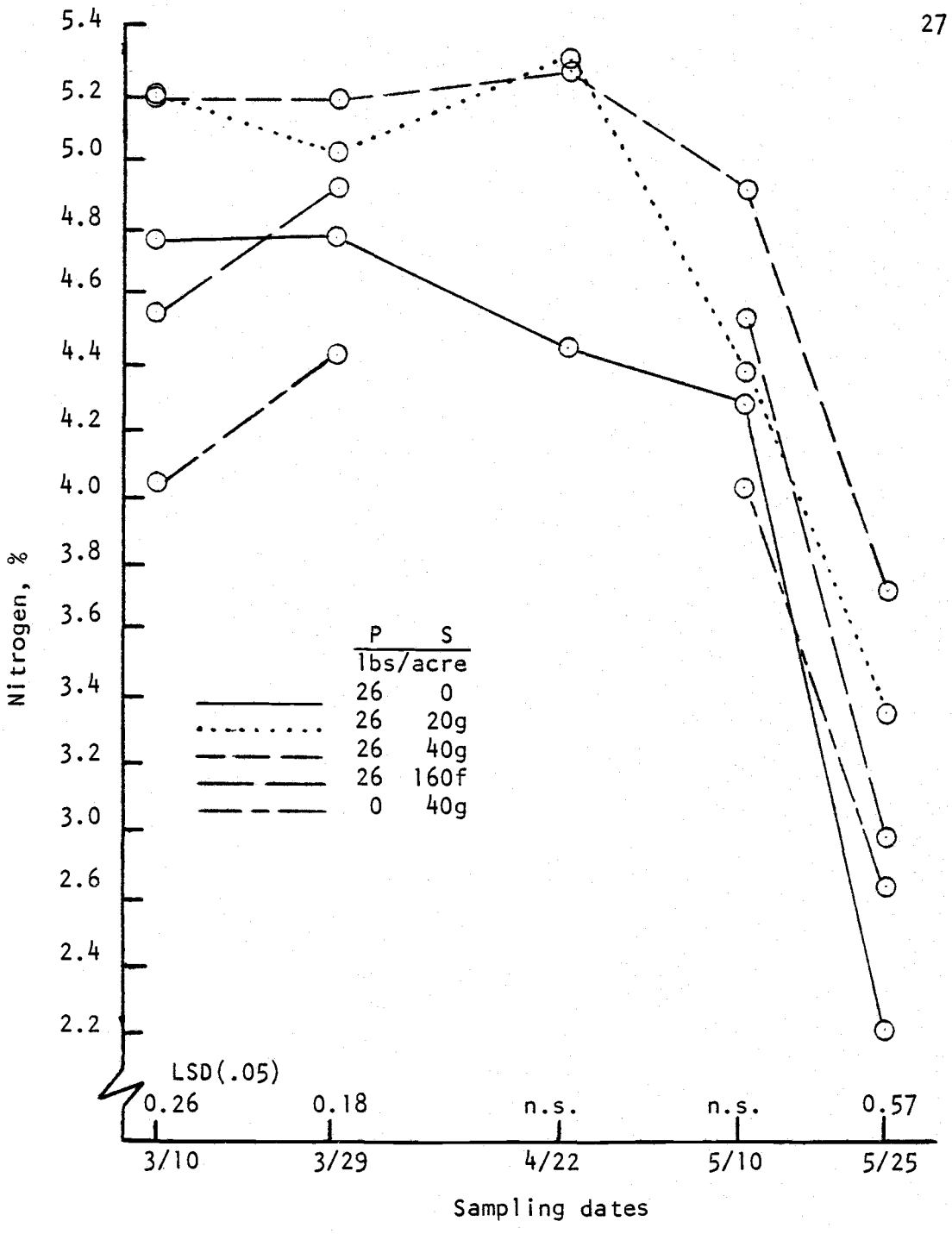

Figure 6. Nitrogen concentration in subterranean clover as influenced by P and S fertilization and plant maturity in 1976. (Sulfur applied as g-gypsum or f-elemental S)

that received 26 P + 20 Sg or 26 P + 160 Sf. Nitrogen concentrations in plants receiving 20 Sg but no P were similar to those receiving 26 P but no S, which indicated that both elements were necessary for maximum N fixation.

In 1977, as in 1976, plants that received only 26 P contained approximately 4.2% N in late March and 3.0% N on May 12 (Figure 7). Significantly higher concentrations, approximately 5.2% N in March and 4.0% N on May 12, were found in clover receiving 26 P plus 20, 40, or 160 S. Plants receiving 40 S but no P contained significantly lower concentrations of N than those receiving the same S treatment plus P.

In both years, the $(N:S)_t$ ratios of clover that did not receive S were 1 to 11 units higher than those receiving 40 or more pounds of S per acre. In 1976, the $(N:S)_t$ for plants receiving 26 P but no S ranged from 22 to 35 during the sampling period (Figure 8). Plants receiving 26 P plus 40 or 160 S or 40 S without P all had similar values ranging from 16 to 27. The ratios from the 26 P + 20 Sg were generally intermediate at each sampling date. There was no satisfactory explanation for the decline on April 22 in the ratio of the clover receiving 26 P but no S; however, only two replications were sampled on this date.

In 1977, the $(N:S)_t$ ratios for clover receiving 26 P but no S ranged from 29 to 35 (Figure 9). These values exceeded those of other treatments at each sampling date. Ratios from 21 to 26 occurred in clover receiving the 26 P + 20 Sg treatment, while clover receiving 160 S with P or 40 S with or without P had the lowest values, ranging from 18 to 24. In general, clover containing total N and S in a ratio greater than approximately 28 at any sampling date were S deficient, while those with lower ratios had adequate S nutrition. These data suggest the value of 28 as the critical $(N:S)_t$ ratio for subterranean clover.

The amount of N left in the residue after extraction varied somewhat with the procedure (Table 8); ethanol extraction left 76 to 87% of the total N in the insoluble portion, while TCA extraction left

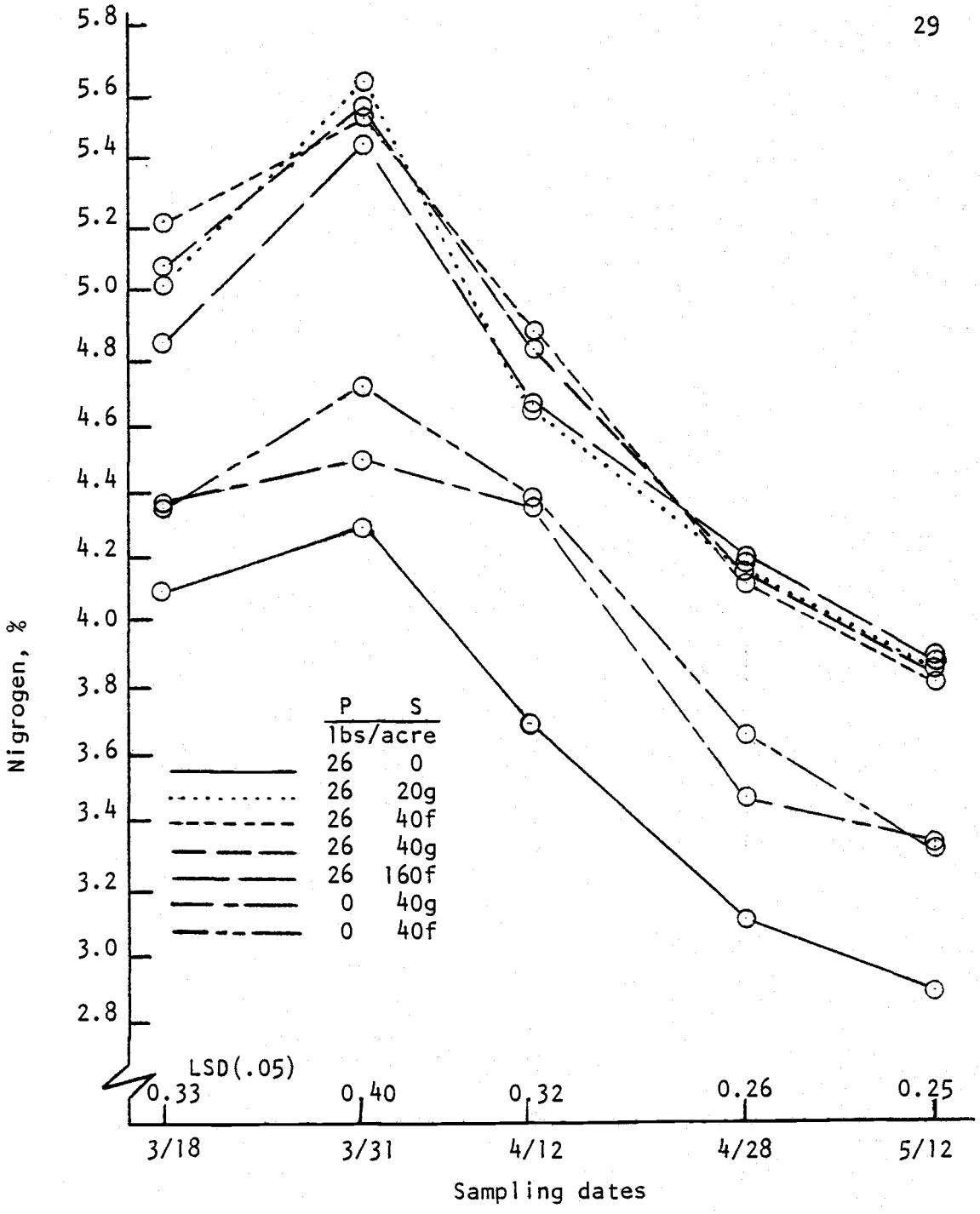

Figure 7. Nitrogen concentration in subterranean clover as influenced by P and S fertilization and plant maturity in 1977. (Sulfur applied as g-gypsum or f-elemental S)

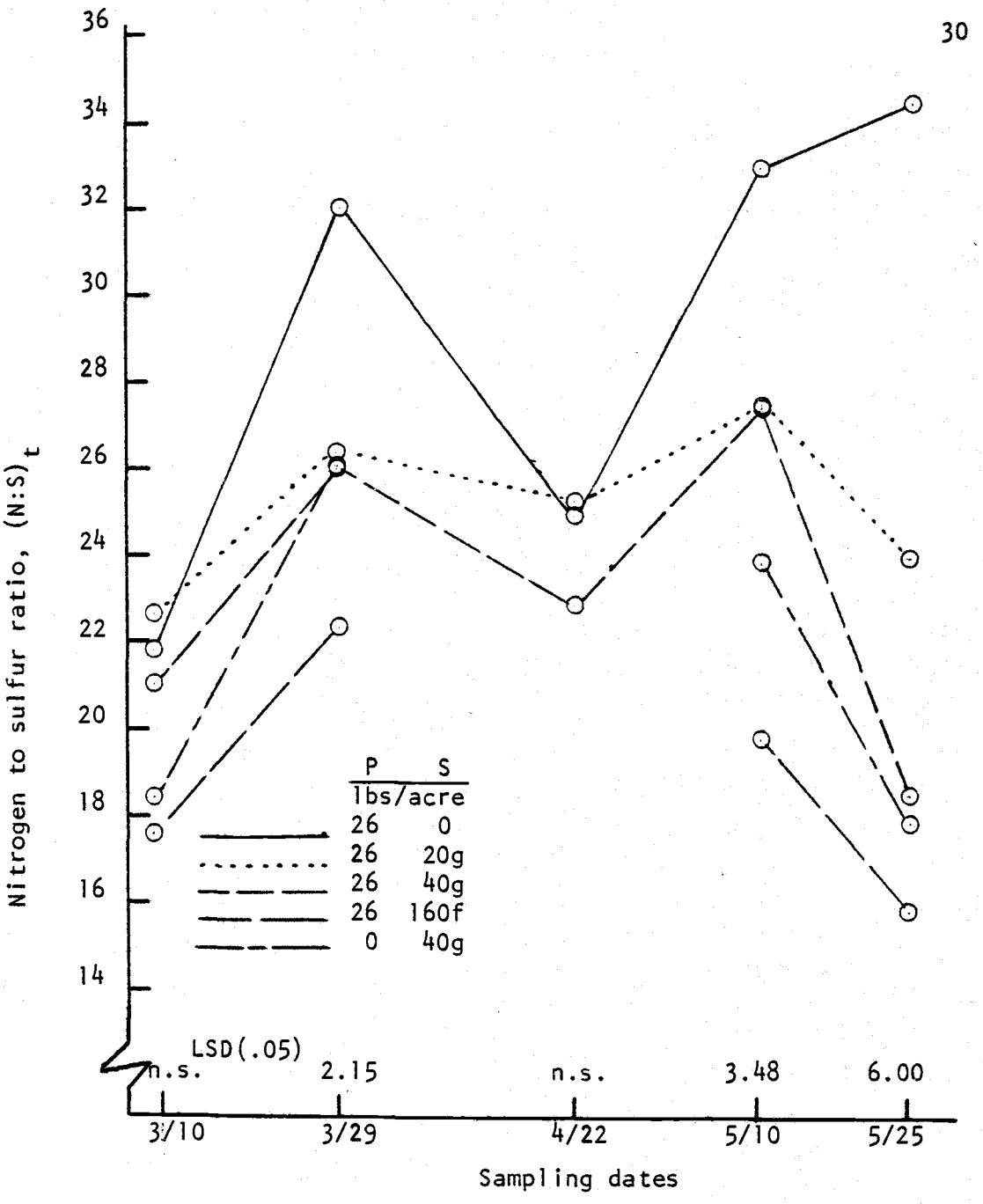

Figure 8. Total nitrogen to total sulfur, $(N:S)_t$, ratios in subterranean clover as influenced by P and S fertilization and plant maturity in 1976. (Sulfur applied as g-gypsum of f-elemental S)

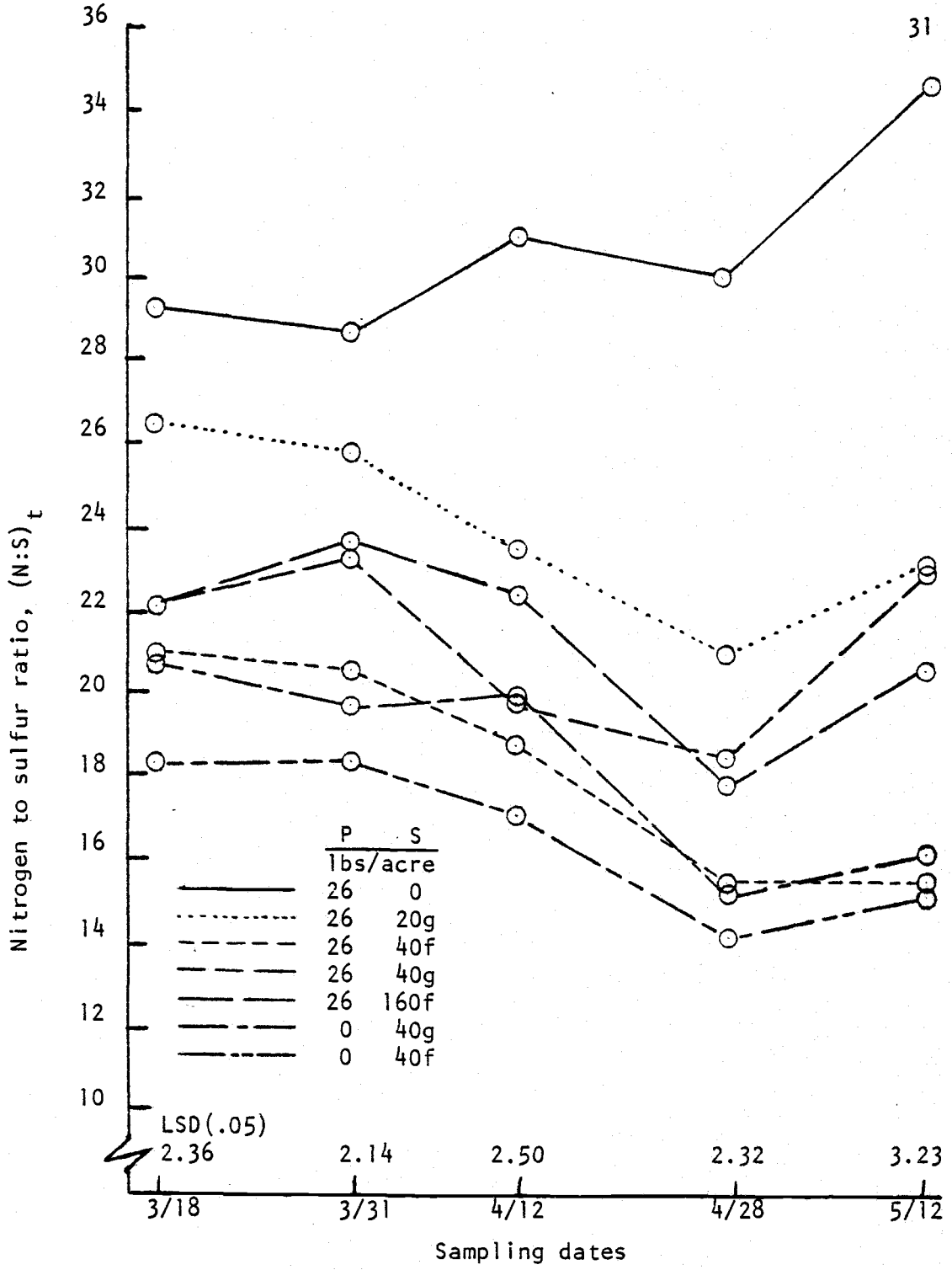

Figure 9. Total nitrogen to total sulfur, $(N:S)_t$, ratios in subterranean clover as influenced by P and S fertilization and plant maturity in 1977. (Sulfur applied as g-gypsum or f-elemental S)

Table 8. Nitrogen and sulfur fractions in subterranean clover as influenced by P and S fertilizer and plant maturity in 1977.

Sampling Date	Treatment lbs/acre		Total			Ethanol-insoluble			TCA-insoluble			Ethanol soluble N	TCA soluble N
	P	S†	N %	S %	N:S	N %	S %	N:S	N %	S %	N:S	%	%
3/18	26	0	4.01	0.16	25.1	3.40	0.10	34.0	3.57	0.10	35.7	0.61	0.44
	26	20g	4.99	0.23	21.7	4.30	0.13	33.1	4.35	0.13	33.5	0.69	0.64
	26	40f	5.15	0.27	19.1	4.50	0.15	30.0	4.54	0.12	37.8	0.65	0.61
3/31	26	0	4.21	0.17	24.8	3.38	0.11	30.1	3.64	0.11	33.1	0.83	0.57
	26	20g	4.98	0.21	23.7	4.13	0.11	37.5	4.20	0.12	35.0	0.85	0.78
	26	40f	5.41	0.26	20.8	4.31	0.16	26.9	4.34	0.12	36.1	1.10	1.07
4/12	26	0	3.74	0.14	26.7	2.99	0.10	29.9	3.18	0.09	35.3	0.75	0.56
	26	20g	4.55	0.20	22.8	3.72	0.12	31.0	3.89	0.10	38.9	0.83	0.66
	26	40f	4.97	0.26	19.1	3.99	0.15	26.6	4.22	0.11	38.4	0.98	0.75
4/28	26	0	3.46	0.13	26.6	2.91	0.09	32.3	3.05	0.08	38.1	0.55	0.41
	26	20g	4.15	0.19	21.8	3.35	0.12	27.9	3.66	0.10	36.6	0.80	0.49
	26	40f	4.49	0.29	15.5	3.80	0.17	22.4	4.01	0.10	40.1	0.69	0.48
5/12	26	0	3.48	0.12	29.0	2.63	0.06	43.8	2.87	0.08	35.9	0.85	0.61
	26	20g	3.87	0.18	21.5	3.22	0.10	32.2	3.34	0.10	33.4	0.65	0.53
	26	40f	4.04	0.27	15.0	3.28	0.14	23.4	3.51	0.10	35.1	0.76	0.53

†g sulfur applied as gypsum
f sulfur applied as fine elemental S less than 0.25 mm in diameter

from 80 to 89%. The absolute values of the ethanol-insoluble portion ranged from 2.63 to 3.40% N in plants that received 26 P but no S and 3.28 to 4.50% N in plants that had received 26 P + 40 Sf. Corresponding values for the TCA-insoluble fraction were 2.87 to 3.64% N and 3.51 and 4.54% N. The absolute values paralleled the total N values, increasing with added S and decreasing with plant maturity.

The soluble N fraction was estimated by subtracting N in the insoluble portion from the total N. Values for the soluble fraction ranged from 0.55 to 1.10% N (mean 0.77% N) in the ethanol extract and 0.41 to 1.07% N (mean 0.61%N) in the TCA extract. The variations in values did not correspond to any sampling date or treatment effects.

The concentration of ammonium-N extracted from the clover with acid varied from 0.04% to 0.10% of the plant with a mean value of 0.07%. The nitrate-N concentration ranged from 0.02 to 0.06% with a mean value of 0.04%. Neither ammonium or nitrate N made up a significant amount of the total N in these samples.

The amount of S left in the residue also varied with the extraction procedure (Table 8). Both extractants removed an average of 36% of the total S from the plants that received no added S. Trichloroacetic acid removed more S from the plants with high S status than did ethanol. Extraction with TCA removed an average of 60% of the total S in the plant, while extraction with ethanol removed an average of 43%. The clover contained a large proportion of the total S in a soluble (presumably nonprotein) form regardless of S nutrition or stage of maturity. Plants with high S status contained a somewhat larger proportion of the total S in a soluble form than did plants of low S status.

The nitrogen to sulfur ratio in the ethanol-insoluble fraction of the clover ranged from 22.4 to 30.0, and from 29.9 to 43.8, for plants that did and did not receive added S, respectively. In the TCA-insoluble fraction, the corresponding ratios ranged from 33.1 to 38.1 and 35.1 to 40.1, respectively. Any variation in $(N:S)_p$ ratios

due to sampling date or S nutrition could not be identified due to the discrepancy between the results of the two extraction procedures.

The N and S relationships revealed by this study were not consistent with relationships previously reported in the literature. The suggested critical (N:S) ratio for subterranean clover of 28 was higher than critical levels previously proposed for other legume tissue. Metson and Collie (1972) reported ratios of 13 to 18 in red clover that did not receive S and 8 to 17 in those that did receive S. In white clover, control plants had $(N:S)_t$ ratios of 23, while plants receiving S had ratios near 17 (Spencer 1959). The critical level for alfalfa in Idaho was 17 to 18 (Westermann 1975a) and 11 in Oregon (Pumphrey and Moore 1965b).

According to Westermann (1975a), the $(N:S)_t$ increased above the $(N:S)_p$ when plants were S deficient because a) protein synthesis was limited by lack of S and nonprotein forms of nitrogen accumulated and b) nonprotein S decreased. However, in subterranean clover a) the $(N:S)_p$ ratio was as high or higher than the $(N:S)_t$, b) even though protein synthesis was limited by S deficiency, extractable (nonprotein) N did not accumulate, and c) extractable (nonprotein) S did not decrease with increasing S deficiency.

The approximate $(N:S)_p$ ratios in subterranean clover, 30 to 35, were much higher than previously reported values for legumes. Walker and Adams (1958) reported $(N:S)_p$ ratios for mixed clover of 17 to 18 in the control and 11 to 17 in plants receiving S. Metson and Collie (1972) reported a range in $(N:S)_p$ from 16.4 to 21.0 in two harvests of white clover. Westermann (1975a) found a ratio of 23 in alfalfa not receiving S and 17 to 18 in plants that did receive S. In a review of published literature, Dijkshoorn (1967) concluded that legumes had fairly constant $(N:S)_p$ ratios approaching 17.5.

The ethanol-soluble and TCA-soluble fraction of plants includes amino acids, amides, ammonium-N, and nitrate-N (Fowden 1961). Sulfur deficiency in alfalfa resulted in decreased levels of all amino acids except arginine and aspartic acid, which apparently accumulated in

the soluble fraction of the plant (Mertz et al. 1952). In wheat and sugar beets, soluble N clearly increased with increased S deficiency (Stewart 1969).

Other researchers have found cases where the ethanol-soluble (nonprotein) N did not increase with increasing S deficiency. In white clover, Metson and Collie (1972) found no evidence of an increase in soluble N with increasing S deficiency. Spencer (1959) found an increase in protein N proportional to total N with increased S supply. Walker and Adams (1958) found that the soluble N in a mixture of subterranean, alsike, white, and red clover remained very constant (about 1.0% of the forage) at different rates of added S. Jones et al. (1971), working with Townsville stylo, found that with moderate S deficiency there was little or no change in $N_p:N_t$. This proportion was affected only with very severe S deficiency.

A previous study (Spencer 1959), showed that essentially all (99%) of the S in S deficient white clover was bound in protein. In contrast, S deficient subterranean clover in this experiment contained an average of 36% extractable S.

Analytical values dependent on protein separation must be defined in terms of the extraction procedure used. Ethanol and TCA are both widely used for separating protein (Bisset 1954; Fowden 1961). Stewart and Street (1946) found that these chemicals extracted comparable amounts of N from plant material.

Extraction times and temperatures varied among researchers. Spencer (1959) extracted with ethanol at room temperature for one hour, Metson and Collie (1972) used ethanol boiling for 3.5 minutes, and Stewart and Whitfield (1965) washed the sample three times with hot ethanol. Metson and Collie (1972) found that boiling the sample 3.5 minutes with ethanol did not extract all of the nonprotein S. Westermann (1975a) concluded that boiling plant material 9 minutes with ethanol did extract all of the nonprotein S.

Jones et al. (1971) and Steward and Street (1946) reported that treatment of samples before extraction could alter the composition of the protein or its behavior with respect to extractants. Some

researchers froze or refrigerated samples (Mertz and Matsumoto 1956), while others dried them at 55°C (Westermann 1975a) or 70°C (Spencer 1959).

It is unlikely that the results reported in this paper were an artifact of the sample treatment or extraction procedures, since previous researchers used similar techniques. However, time available for this study did not allow a thorough evaluation of protein separation techniques.

The proportion of soluble (nonprotein) N and insoluble (protein) N appeared to correspond to previous results for Trifolium species, but the $(N:S)_p$ was approximately two times higher. The only N:S ratio in subterranean clover that approached the $(N:S)_p$ ratio values found in other legumes was $N_p:S_t$ (insoluble N to total S). Equating $(N:S)_p$ in other legumes to $N_p:S_t$ in subterranean clover would require the assumption that both extraction procedures had preferentially removed S from the protein while N remained in the residue after extraction.

If the analytical results were valid, two conclusions about subterranean clover can be made:

1) immature subterranean clover contains a pool of soluble (nonprotein) S that must be satisfied before S is available for protein synthesis, and
2) immature subterranean clover has a lower percentage of S in the protein than other legumes.

Much of the previously reported $(N:S)_p$ data was from whole plant tops collected at hay stage. The fact that subterranean clover leaflets and petioles were analyzed at immature stages of growth may be responsible for some of the differences between $(N:S)_p$ ratios found in this plant material and those reported for other plants. It is known that the amino acid composition (thus the N and S content) of the whole or "bulk" proteins in plants are much more alike than the amino acid composition of particular proteins (Fowden 1961). Theoretically, particular parts of the plant at

different stages of growth could have different functions which would require different proteins. Data from Jones et al. (1971) did show a change in $(N:S)_p$ in Townsville stylo with maturity; however, analysis of the leaves of 113 day old white clover leaves (Spencer 1959) showed $(N:S)_p$ ratios between 16 and 20, much lower than that of subterranean clover leaves and petioles.

The results from this experiment suggested that further investigations be conducted to study the nature of the protein and nonprotein fractions of subterranean clover.

SUMMARY

Field experiments were established to evaluate the use of plant analysis to predict response of subterranean clover to P and S fertilization. Data collected included yield at harvest, P, S, and N concentrations of grass and clover forage at harvest, and P, S, and N analyses of clover (leaflets and petioles) collected at five or six sampling dates during the spring growing seasons of 1976 and 1977.

Application of 2 tons of lime plus 17 or 51 pounds of P per acre to subterranean clover grown on the Veneta soil resulted in a yield increase of 28%, while application of 26 pounds of P per acre to clover on the Nonpareil-Oakland soil resulted in yield increases ranging from 22 to 67%. On both sites, P concentrations in the mixed grass and clover forage at harvest were higher in plants that had received P fertilization (0.20%) than in plants that had not (0.14%). The P concentration of clover that had received P fertilization exceeded that of clover that had not at every sampling date both years. The P concentration of clover not receiving P was approximately 0.22% from mid-March to mid-April and dropped to 0.18% in mid-May, while P-fertilized clover contained 0.28% P from mid-March to mid-April and 0.25% P in mid-May. The P concentration of clover appeared unaffected by lime rates from zero to four tons/acre and S applications from zero to 160 pounds per acre. The P concentration of all plants remained relatively stable from mid-March to mid-April, regardless of treatment. The P critical level for subterranean clover leaflets and petioles collected between mid-March and mid-April was 0.25% P.

Sulfur fertilization on the Nonpareil-Oakland soil complex increased yield more than 45%. Applications of 40 or more pounds of S per acre were adequate for maximum yield and more than doubled the S concentration of the mixed grass and clover forage in both 1976 and 1977. The sulfur concentration of the clover increased with S fertilization at every sampling date. In late March, the controls

contained approximately 0.15% S and the S-treated plants contained 0.22% S, while in mid-May the controls contained 0.08% S and the S-treated plants contained 0.20% S. Sulfur concentration appeared relatively stable from mid-March to mid-April. A concentration of 0.20% S was identified as the critical level for clover samples collected during this time.

The $(N:S)_t$ ratio in S deficient clover ranged from 21 to 35 during two growing seasons, while the $(N:S)_t$ ratios of clover receiving S fertilization adequate for maximum yield ranged from 16 to 27. The apparent critical $(N:S)_t$ ratio was 28.

The N:S ratio in the insoluble (protein) fraction of the clover, estimated using ethanol and TCA extractions, was approximately 30 to 35 from March 18 to May 12 in 1977. The ethanol-soluble and TCA-soluble (nonprotein) N concentration remained fairly constant at about 80% of the total N regardless of the level of S fertilization or stage of maturity. Ammonium-N and nitrate-N, determined using a formic acid extraction, comprised about 0.07% and 0.04% of the plant, respectively.

Ethanol and TCA extracted different amounts of S from the clover. Ethanol extracted an average of 43% of the total S from plants receiving S adequate for maximum yield, while TCA extracted an average of 60% from the same samples. Both procedures removed an average of 36% of the total S from S deficient clover. These results indicated either the presence of nonprotein S in S deficient and adequate clover at all sampling dates or that these procedures extracted proportionately more S than N from the protein fraction of the plants.

The problems encountered evaluating protein and nonprotein N and S in these samples suggested that further research be conducted to investigate the relationships between protein synthesis and the accumulation of nonprotein N and S in subterranean clover, as well as the chemical procedures necessary to evaluate these processes. If these extractions gave a true indication of the proportion of protein and nonprotein N and S, subterranean clover has a $(N:S)_p$ ratio wider than the 17.5 value reported for other legume tissue.

The generalizations about N:S ratios in legume tissue may need to be reevaluated in terms of particular species, plant parts, and stage of maturity.

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APPENDICES

APPENDIX A

Plant Analysis for Sulfur

A 0.5 g sample of plant material was weighed into a 50 ml beaker with 2 mls of 95% ethanol and 3.5 mls of concentrated $Mg(NO_3)_2$. The sample was warmed on low heat until the ethanol was driven off, then dry ashed at 500°C for 5 hours. After the sample cooled to room temperature, 10 mls of 3N HCl were added. The solution was filtered into a 50 ml volumetric flask and diluted to volume with double distilled water.

A 10 ml aliquot of the digested sample was placed in a plastic vial with 1 ml of an acid solution consisting of 50% concentrated HCl and 50% glacial acetic acid. The vial was placed in a constant temperature room at 20°C for at least an hour. Then, 1 ml of a solution, consisting of 0.6 g of gelatin and 4 g of $BaCl_2$ in 200 mls of water, was added. After 30 minutes, but not more than 60 minutes, the sample was swirled to insure mixing and the absorption (turbidity) read on a spectrophotometer at 500nm.

The sample absorption was compared to a standard curve to determine concentration of S.

APPENDIX B

Table B-1. The nutrient concentration of subterranean clover grown on the Nonpareil-Oakland soil complex as affected by P and S fertilizer and plant maturity in 1976.

Treatment			Sampling date				
	P	S†					
	lbs/acre		3/10	3/29	4/22	5/10	5/25
			K, %				
1.	26	0	2.0	2.2	2.2	2.3	2.8
2.	26	20g	1.8	1.8	2.2	2.0	2.3
3.	26	40g	1.8	1.8	1.8	1.6	2.2
5.	26	160f	1.8	1.4	---	1.5	1.6
7.	0	40g	2.2	2.0	---	2.2	2.0
			Mn, ppm				
1.	26	0	143	118	80	103	87
2.	26	20g	122	106	87	102	103
3.	26	40g	120	99	97	88	132
5.	26	160f	133	135	---	124	153
7.	0	40g	139	102	---	90	89
			Ca, %				
1.	26	0	---	---	1.30	---	---
			Mg, %				
1.	26	0	---	---	0.29	---	---
			Zn, ppm				
1.	26	0	---	---	37	---	---
			Cu, ppm				
1.	26	0	---	---	13.2	---	---

†g sulfur applied as gypsum
f sulfur applied as elemental S

Table B-2. The nutrient concentration of subterranean clover grown on the Nonpareil-Oakland soil complex as affected by P and S fertilizer and plant maturity in 1977.

Treatment			Sampling date				
	P	S†					
	lbs/acre		3/18	3/31	4/12	4/28	5/12
			K, %				
1.	26	0	2.2	2.6	3.0	2.9	2.8
2.	26	20g	1.9	2.0	2.2	1.9	1.9
3.	26	40g	2.0	2.1	2.1	2.1	2.0
4.	26	40f	1.9	1.9	2.1	2.0	1.9
5.	26	160f	2.0	1.9	2.2	2.0	1.6
7.	0	40g	2.2	2.6	3.0	2.9	2.6
8.	0	40f	2.2	2.6	3.0	2.8	3.0
			Ca, %				
1.	26	0	1.08	1.18	1.22	1.27	1.57
2.	26	20g	1.07	1.14	1.15	1.33	2.02
3.	26	40g	1.06	1.14	1.12	1.24	1.83
4.	26	40f	1.02	1.06	1.06	1.20	1.77
5.	26	160f	1.03	1.15	1.16	1.30	1.93
7.	0	40g	0.96	1.10	1.11	1.42	1.67
8.	0	40f	0.87	0.98	1.04	1.11	1.53
			Mg, %				
1.	26	0	0.36	0.36	0.29	0.37	0.42
2.	26	20g	0.35	0.34	0.30	0.37	0.49
3.	26	40g	0.33	0.34	0.31	0.37	0.45
4.	26	40f	0.32	0.32	0.29	0.36	0.47
5.	26	160f	0.33	0.34	0.31	0.37	0.48
7.	0	40g	0.31	0.30	0.20	0.30	0.38
8.	0	40f	0.29	0.29	0.22	0.28	0.38
			Zn, ppm				
1.	26	0	53	50	43	45	52
2.	26	20g	48	39	33	33	46
3.	26	40g	50	41	35	36	47
4.	26	40f	51	44	38	38	51
5.	26	160f	54	44	36	36	31
7.	0	40g	56	52	46	46	59
8.	0	40f	55	53	46	47	61

Table B-2, Continued.

Treatment			Sampling date				
	P	S†					
	lbs/acre		3/18	3/31	4/12	4.28	5.12
			Cu, ppm				
1.	26	0	16	14	14	14	10
2.	26	20g	17	15	16	17	15
3.	26	40g	16	15	16	17	14
4.	26	40f	15	15	16	16	14
5.	26	160f	17	16	16	17	14
7.	0	40g	19	17	17	17	13
8.	0	40f	19	17	17	16	13
			Mn, ppm				
1.	26	0	163	152	154	128	152
2.	26	20g	145	124	110	110	157
3.	26	40g	133	123	109	108	148
4.	26	40f	162	153	142	134	176
5.	26	160f	169	154	130	121	174
7.	0	40g	157	127	124	110	157
8.	0	40f	170	151	130	133	180

†g sulfur applied as gypsum
f sulfur applied as fine elemental S less than 0.25 mm in diameter

Table B-3. The nutrient concentration of subterranean clover grown on Veneta silt loam as affected by lime and P treatments and plant maturity in 1977.

Treatment		Sampling date				
lime tons/acre	P lbs/acre	3/18	3/31	4/12	4/28	5/12
		K, %				
0	0	2.4	2.9	3.3	2.6	2.8
0	17	2.5	3.0	3.0	2.5	2.7
0	51	2.4	2.2	2.5	2.2	2.1
2	0	2.2	2.4	2.8	2.2	2.2
2	17	2.4	2.4	2.3	1.9	1.8
2	51	2.1	2.0	2.3	1.7	1.8
4	0	2.4	2.5	2.8	2.1	2.1
4	17	2.4	2.5	2.8	2.0	2.0
4	51	2.6	2.4	2.3	1.8	1.6
		Ca, %				
0	0	1.01	1.30	1.27	1.42	1.71
0	17	1.05	1.12	1.10	1.33	1.56
0	51	1.10	1.14	1.05	1.17	1.66
2	0	1.23	1.42	1.52	1.75	2.24
2	17	1.55	1.63	1.62	1.87	2.35
2	51	1.51	1.55	1.53	1.92	2.35
4	0	1.23	1.40	1.57	1.70	2.14
4	17	1.42	1.60	1.63	1.96	2.34
4	51	1.57	1.54	1.51	2.01	2.20
		Mg, %				
0	0	0.24	0.30	0.28	0.31	0.39
0	17	0.27	0.28	0.28	0.35	0.44
0	51	0.27	0.26	0.28	0.34	0.44

Table B-3, Continued.

Treatment		Sampling date				
lime tons/acre	P lbs/acre	3/18	3/31	4/12	4/28	5/12
		Mg, % cont.				
2	0	0.24	0.24	0.28	0.30	0.38
2	17	0.28	0.29	0.28	0.34	0.40
2	51	0.27	0.28	0.30	0.36	0.43
4	0	0.25	0.26	0.31	0.31	0.39
4	17	0.28	0.30	0.28	0.35	0.42
4	51	0.31	0.31	0.31	0.37	0.42
		Zn, ppm				
0	0	37	35	33	34	42
0	17	35	34	34	37	44
0	51	42	34	36	36	46
2	0	27	22	26	26	30
2	17	26	20	25	26	26
2	51	25	24	22	26	27
4	0	27	22	26	29	34
4	17	25	20	24	26	31
4	51	24	18	20	25	28
		Mn, ppm				
0	0	147	186	162	163	239
0	17	187	206	198	212	287
0	51	200	212	199	213	296
2	0	125	157	129	136	188
2	17	122	184	159	167	208
2	51	118	143	110	134	176
4	0	132	148	122	134	164
4	17	108	117	120	137	181
4	51	121	149	134	157	216